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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 6.

THE CONSTITUTION OF LAMINARIN. ISOLATION OF 2 : 4 : 6–TRIMETHYLGLUCOPYRANOSE.

By VINCENT C. BARRY, M.Sc.,

Chemical Laboratories, University College, Galway.

[Read December 13, 1938. Published separately January 25, 1939.]

In a previous communication (1) laminarin has been shown to be a polysaccharide built up exclusively of glucose units, and occurring as an unstable colloid whose rotatory power varies with the strength of its aqueous solution. Evidence has also been given (*loc. cit.*) which indicated the possibility that laminarin occurs in the plant as an ethereal sulphate complex.

Based on the remarkable property it has of spontaneous precipitation, a simple method of obtaining it in a pure state has been described. The present communication deals first with attempts made at isolating the laminarin sulphate undecomposed, and secondly with the preparation and properties of acetyl and methyl derivatives of the polysaccharide. By a study of the hydrolytic products of the latter, the author is able to suggest a constitutional formula for laminarin.

Laminarin may be extracted from the fronds of laminariae by cold water, and the aqueous extract, on standing with dilute hydrochloric acid, gives a characteristic flaky deposit of the polysaccharide. Simultaneously with the deposition of laminarin, there is an increase in the sulphate ions in solution. This information, taken in conjunction with the fact that laminarin deposited from aqueous solution is practically insoluble in cold water, has led the author to suggest (*loc. cit.*) that the polysaccharide occurs in the plant as an ethereal sulphate. Extraction of the weed in an acid medium seems to leave behind the fucoidin, itself an ethereal sulphate (2). As the hydrochloric acid employed in the preparation of laminarin would lead to the splitting off of the sulphuric acid residue, even if extraction had taken place without decomposition, it was sought, by replacing the mineral acid by very dilute oxalic acid, to extract the ethereal sulphate undecomposed. Oxalic acid extracts of various strengths were used, the weakest being 0·25 per cent. Even with one per cent. oxalic acid the extraction of the fucoidin must have been prevented to a great extent, as after hydrolysis of the purified extract treatment with phenylhydrazine failed to yield any fucosazone. These oxalic acid extracts after purification did indeed contain sulphuric acid, but only to the extent of less than one per cent. of the laminarin. It may be that the addition of baryta or dilute sulphuric acid during purification is sufficient to hydrolyse the ethereal sulphate, and that the combined sulphuric acid found represents a residue of undecomposed laminarin sulphate. On the other hand, it may be due to contamination with

a little fucoidin. The experiments cannot, therefore, be regarded as affording satisfactory evidence of the existence in the plant of an ethereal sulphate of laminarin.

The laminarin isolated in the above manner was finally separated from aqueous solution by the addition of alcohol. It separated in a very fine colloidal condition, and when dried dissolved easily in cold water. On the other hand, laminarin which is separated by spontaneous deposition is almost insoluble in cold water. This difference in solubility could be accounted for by the extremely fine state of division of the particles precipitated by alcohol or by the existence in the fronds of another modification of the polysaccharide with possibly a smaller molecule (3, 4, 5). When, however, the laminarin which dissolves in cold water is allowed to stand in dilute aqueous solution to which a drop of hydrochloric acid has been added, spontaneous deposition takes place, and flakes of laminarin separate which can be washed with cold water. Complete acetylation of these modifications yields acetates similar in properties, and having the same specific rotatory power. The essential difference, therefore, between the two products lies only in the size of the colloidal particles. Further evidence has thus been obtained that only one laminarin occurs in seaweed, and that the differences observed between various extracts are due to the remarkable property possessed by laminarin of assuming different colloidal forms.

Laminarin may be acetylated with acetic anhydride and fused sodium acetate to a triacetate. The acetylation proceeds more smoothly, however, when carried out by the method devised by Barnett (6), and applied to glycogen by Haworth, Hirst, and Webb (7). Saponification of the triacetate with alkali gave a solution of laminarin, which, when made faintly acid, became milky in a few days, and gave the characteristic flaky deposit. The fact that laminarin recovered in this way retains its property of spontaneous precipitation indicates that very little degradation of the molecule can have taken place during the acetylation.

When the laminarin triacetate was hydrolysed with methyl alcoholic hydrogen chloride a syrup was obtained which crystallised immediately on addition of absolute alcohol. The crystals were pure α-methyl glucoside.

It was sought at first to carry out the methylation of the laminarin acetate in acetone with methyl sulphate and 30 per cent. caustic soda at 50° C. This was the method used by Haworth, Hirst, and Webb (7) for the methylation of glycogen acetate. Although 29 per cent. methoxyl was introduced in one treatment, the product was excessively dirty, and it was thought best to methylate at ordinary temperatures, using 45 per cent. potash. The latter method gave, with six or seven treatments with the reagents, a product containing 44·7 per cent. methoxyl. The theoretical value for trimethyl laminarin is 45·6 per cent.

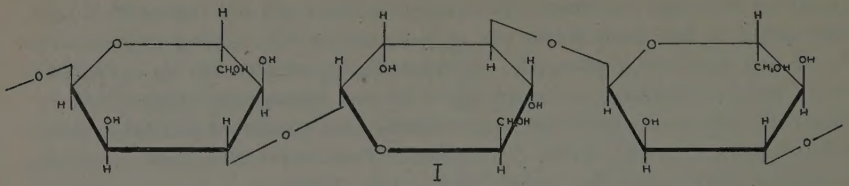
The hydrolysis of the trimethyl laminarin was carried out with 2 per cent. methyl alcoholic hydrogen chloride. The usual treatment gave a syrup, which

was distilled in a high vacuum. The syrupy distillate did not reduce Fehling's solution until it had been boiled for a few minutes with strong hydrochloric acid. It was, therefore, a glucoside. It crystallised partially, but the crystalline fraction did not increase on standing. It was accordingly hydrolysed by boiling with aqueous hydrochloric acid. Subsequent treatment yielded a syrup which crystallised readily from dry ether. Two recrystallisations from the same solvent gave colourless needles, M.P.: 113–114° C.

It was thought at first that these crystals were 2 : 3 : 6 trimethyl glucose, which is frequently obtained with a melting point about this temperature, and which is the trimethyl glucose resulting from the hydrolysis of methylated starch, cellulose, glycogen, etc. However, a mixed melt with an authentic specimen of 2 : 3 : 6-trimethyl glucopyranose obtained from Professor W. N. Haworth, F.R.S., showed a big depression. Also the specific rotatory power of the crystals in methyl alcoholic hydrogen chloride showed no inversion of sign after ten days' standing. This inversion of sign is characteristic of the 2 : 3 : 6 variety (8). Finally, a mixed melt with 2 : 4 : 6-trimethyl glucopyranose, also supplied by Professor Haworth, showed no depression. The rotation data for the crystals were also similar to those recorded by Lake and Peat (9) for this trimethyl glucose.

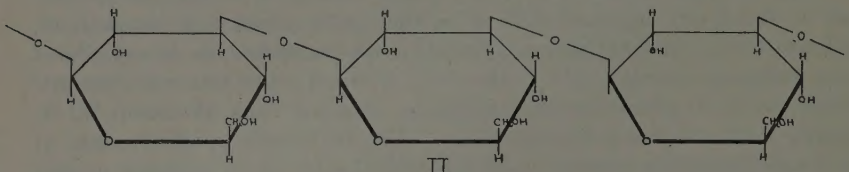
Allowing for slight mechanical loss, laminarin can be quantitatively converted into trimethyl laminarin. The latter on hydrolysis yields a trimethyl glucopyranose with the methyl groups in the positions 2, 4, and 6. Although the yield of the trimethyl glucose is not yet available, no other trimethyl glucose was encountered. The presumption is thus that in laminarin the hexose units are attached to each other through positions 1 and 3 (I or II in the figure), and not, as in cellulose, lichenin, starch, and glycogen, through positions 1 and 4 (III or IV). Zechmeister and Toth (10) have isolated from yeast membrane a glucose polysaccharide which after methylation and hydrolysis yielded also 2 : 4 : 6 trimethyl glucose. The same linkage, therefore, occurs in both these polysaccharides.

Haworth (11) has pointed out that a chain of β-glucopyranose units (IV) with a 1, 4 linkage forms a more symmetrical structure than a chain of similarly linked α-glucopyranose units (III), and suggests that the crystalline character of cellulose is related to this symmetrical structure. With the 1, 3 linkage, however, it will be seen that α-glucopyranose units form a more symmetrical structure (I) than β-glucopyranose units (II). On the other hand, while α-glucopyranose units arranged as in (I) form a somewhat zig-zag but direct extended chain, β-glucopyranose units as arranged in (II) will tend to curve into a ring or more probably a spiral. A spiral structure of the latter kind would explain, by the entanglement of the molecules which must take place, the ease with which laminarin aggregates in aqueous solution, and the spontaneous precipitation which it undergoes. If the correctness of this reasoning is allowed, it appears probable that laminarin will be found to consist of a chain of β-glucopyranose units linked as shown, and bent into a spiral form.



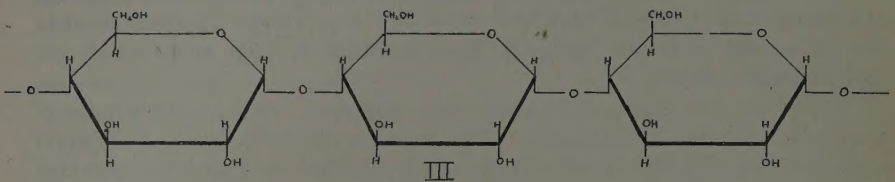
I

ILLUSTRATION OF 1,3 LINKAGE WITH α-GLUCOPYRANOSE UNITS



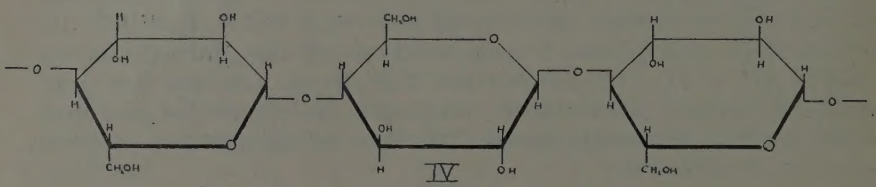
II

ILLUSTRATION OF 1,3 LINKAGE WITH UNITS OF β-GLUCOPYRANOSE



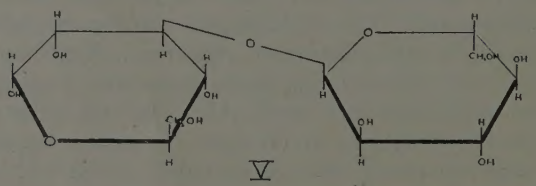
III

FRAGMENT OF STARCH MOLECULE (α-GLUCOPYRANOSE UNITS)



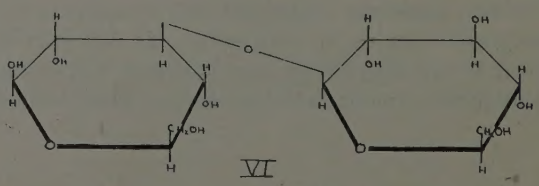
IV

FRAGMENT OF CELLULOSE MOLECULE (β-GLUCOPYRANOSE UNITS)



V

ILLUSTRATING DISACCHARIDE WITH 1,3 LINKAGE OF α-GLUCOPYRANOSE UNITS



VI

DISACCHARIDE WITH 1,3 LINKAGE OF β-GLUCOPYRANOSE UNITS

The following table shows the specific rotatory powers of cellulose, lichenin, starch, glycogen, and laminarin, and those of their acetyl and methyl derivatives :—

	Cellulose.	Lichenin.	Starch.	Glycogen.	Laminarin.
Polysaccharide . . .	− 3·45°*	+ 8·3°† − 2·35°*	+ 190°‡	+ 192°	− 7° to − 16°
Acetyl Derivative (Chloroform)	− 22°	− 38·5°	+ 170°	+ 163°	− 52°
Methyl Derivative (Chloroform)	− 10°	—	+ 208°	+ 208°	− 4·39°

* $[\alpha]^{22°}_{435·8}$ In Cuprammonium. † In 2N . NaOH. ‡ Representative value.

It will be seen that laminarin resembles cellulose and lichenin rather than starch and glycogen. This seems to indicate that laminarin is built up of β-glucopyranose units, as already suggested, and as is the case in the two first-mentioned polysaccharides. The existence, however, of a different linkage in laminarin may complicate this argument from analogy.

Before the trimethyl glucose obtained above was definitely identified as the 2 : 4 : 6 variety, exhaustive attempts were made at isolating cellobiose octacetate by acetolysis of laminarin. Naturally, all these attempts met with failure. It will be clear from the constitution advanced above for laminarin that it should be possible to isolate from it a disaccharide (laminariose) having the structure shown in either V or VI. This possibility is at present being investigated. Zechmeister and Toth (10) have obtained from their polysaccharide a disaccharide in the form of an osazone. This may be the same disaccharide as occurs in the laminarin molecule.

EXPERIMENTAL.

Occurrence of Laminarin. 50 g. of dry fronds of *L. Cloustoni* collected in September, 1937, were cut into small pieces and stirred for 3 days with 500 c.c. dilute oxalic acid [0·25% $(COOH)_2 2H_2O$]. The liquid was very milky. It was decanted, and excess lead acetate solution was added. This produced a copious colloidal precipitate, which was removed by filtration, and the lead was then removed by the addition of dilute sulphuric acid. The acid filtrate was now treated with a large excess of spirit, and the white colloidal precipitate collected on a filter paper. After washing with spirit it was redissolved in distilled water and again thrown down by alcohol. It was collected on a glass filter, and, after washing with alcohol and ether, was dried in a vacuum desiccator over sulphuric acid to a perfectly white powder. This material dissolved easily in cold water to a clear solution, which gave no turbidity with barium nitrate

solution unless it had first been boiled with hydrochloric acid for a few minutes. A thoroughly dried, weighed quantity of it was accordingly heated on a boiling water bath for 2 hours with 5 per cent. aqueous hydrochloric acid. The sulphuric acid in the hydrolysate was precipitated and weighed as barium sulphate. It corresponded to 0·9 per cent. SO_4''. Alterations in the procedure of extraction and purification failed to yield a material containing a greater quantity of combined sulphate.

Properties of the Laminarin Isolated as above. The material isolated according to the above method was a perfectly white powder dissolving easily in cold water. It had $[\alpha]_D^{15°}$, - 12·8° ($c = 3·5$). 1·9 g. of it were dissolved in 25 c.c. of distilled water, and allowed to stand after the addition of one drop of concentrated hydrochloric acid. In 3 days the laminarin had begun to deposit. After 10 days the deposited material weighed 0·4 g., and deposition was going on slowly. The separated laminarin showed a similar variation of rotatory power with concentration to that previously recorded (1).

Acetylation of Laminarin. 1. (With sodium acetate and acetic anhydride.) 3 g. of powdered fused sodium acetate and 30 c.c. acetic anhydride were heated to boiling, and then 4·5 g. of laminarin were added, and the boiling continued for about 10 minutes. The acetylation was completed by heating on a boiling water-bath for 3 hours. The mixture was stirred into a large volume of water, and the acetylated laminarin separated in lumps, which were broken under the water. After standing overnight the acetylated product was filtered through a glass filter, and washed well with distilled water. Purification was effected by precipitation from chloroform solution with petrol ether. After drying in a vacuum desiccator the laminarin acetate was obtained as a light white powder, easily soluble in chloroform and acetone, less soluble in alcohol. It gave a slight reduction of Fehling's solution. Saponification showed it to be a triacetate of laminarin. $[\alpha]_D^{18°}$, in chloroform, - 51·7°.

2. (With chlorine and sulphur dioxide.) 4 g. of pure laminarin were dissolved in distilled water, and reprecipitated by a large volume of alcohol. The laminarin, which separated in a very fine colloidal state, was collected on a glass filter, and washed with alcohol and ether. It was finally dried in a desiccator over sulphuric acid for a few hours. It was now suspended in glacial acetic acid (30 c.c.) through which chlorine had been bubbled for a short time. Acetic anhydride (50 c.c.) containing roughly an equivalent amount of sulphur dioxide was then added. Heat was developed, and the laminarin dissolved completely in a few minutes. After 15 minutes the liquid was poured through a glass filter into a large volume of distilled water. The resulting material was similar to that obtained above. The yield was almost theoretical. $[\alpha]_D^{18°}$, - 52·0° in chloroform. Laminarin triacetate prepared from the material got by the oxalic acid extraction had $[\alpha]_D^{18°}$, - 51·1°. The acetyl content calculated from the alkali consumption was 44·2 per cent. Calculated for laminarin triacetate, 44·8 per cent. As pointed out by Haworth, Hirst, and Webb (7) with regard

to glycogen, acetylation of laminarin which was not previously precipitated by alcohol requires much longer heating, and gives inferior yields.

Recovery of Laminarin from the Acetate. In the determination of the acetyl content of the acetate, as described above, the solution resulting from the saponification was made slightly acid, and allowed to stand. After 2 days the liquid became milky, and laminarin began to deposit. The laminarin recovered in this way, when redissolved in water, deposits spontaneously as before.

α-Methylglucoside from the Acetate. Laminarin triacetate (3 g.) was boiled on a water-bath with 5 per cent. methyl alcoholic hydrogen chloride (100 c.c.) for 11 hours. The acetate dissolved immediately, and after a few minutes the solution became milky. A clear solution was again obtained after 2 hours. The liquid was neutralised with silver carbonate, and after separation of the silver salts the neutral liquid was evaporated to dryness under reduced pressure. A syrup was obtained which crystallised immediately on the addition of absolute alcohol. The crystals were α-methyl glucoside, which after one recrystallisation from the same solvent melted at 165° C. Yield, 1·4 g.

Simultaneous Deacetylation and Methylation of Laminarin Triacetate. As already noted, methylation of the acetate in acetone at 50° C. with 30 per cent. aqueous sodium hydroxide and methyl sulphate produced a dirty product (methoxyl content after one methylation, 29 per cent.). This method was accordingly abandoned.

9 g. of laminarin triacetate in 100 c.c. of acetone were stirred vigorously in a stream of running water, and treated with methyl sulphate (100 c.c.) and 45 per cent. potassium hydroxide (160 c.c.). The addition of the reagents occupied from 2½ to 3 hours. The mixture was stirred for a further 7 hours. Most of the methylated product was out of solution at the close; it was separated by filtration. The filtrate was carefully neutralised and concentrated to get rid of most of the acetone. It was now extracted with chloroform, and the chloroform extract on evaporation gave a further small quantity of methylated product. The combined yields were dissolved in a small volume of chloroform, and precipitated with petrol ether. Two such treatments gave a product containing 39·3 per cent. methoxyl. Two further methylations raised the methoxyl content to 40·5 per cent. In general, 7 methylations were required to raise the methoxyl to 44·7 per cent. When precipitated from chloroform the trimethyl laminarin was obtained as a light, cream-coloured powder, easily soluble in chloroform, less so in acetone, slightly soluble in alcohol, ether, and water. $[\alpha]_D^{18°}$, in chloroform, −4·39°.

3·730 mg. gave 12·530 mg. silver iodide, corresponding to OCH_3, 44·7 per cent.

5·095 mg. gave 9·750 mg. CO_2 and 3·680 mg. H_2O, corresponding to C, 52·21; H, 8·08.

Calculated for trimethyl laminarin: C, 52·90; H, 7·84; OCH_3, 45·6.

The yield was 6·0 g., i.e., 94 per cent. of the theoretical. When obtained by evaporation from chloroform solution, it is got as a colourless or faintly yellow glass. It has a very slight reducing action on Fehling's solution.

Hydrolysis of Trimethyl Laminarin. Trimethyl laminarin (4 g.) was heated to gentle boiling under a reflux condenser with 2 per cent. methyl alcoholic hydrogen chloride (100 c.c.) until the rotation reached a maximum (29 hours). After neutralization with silver carbonate the neutral filtrate was taken to dryness under reduced pressure. A yellow viscid syrup (3·9 g.) resulted. This had no action on Fehling's solution. 3 g. of this syrup were distilled in a high vacuum (0·01 mm.). Nearly all the syrup distilled at 130°–140° C. (bath temperature). The distillate was a colourless syrup (2·5 g.) which partially crystallised on keeping. As there did not appear to be any increase in the crystalline fraction on standing, the whole distillate was subjected to hydrolysis with 5 per cent. aqueous hydrochloric acid. After heating to gentle boiling for three hours the acid was neutralised with barium carbonate, and the neutral liquid evaporated to dryness under reduced pressure. Extraction of the residue several times with boiling dry ether gave, on evaporation of the ether, a syrup which crystallised. Two recrystallisations from the same solvent gave colourless needles, M.P. 113–114° C.; $[a]_D^{12°}$, in water, $+99·3° \rightarrow +74·2°$ (equilibrium value), 2 : 4 : 6 trimethyl glucopyranose melts at 115° and has $[a]_D^{20°}$ in water, $+98·2° \rightarrow +74·8°$ (9).

C, 48·43; H, 8·27; OCH_3, 41·23.
Calculated for trimethyl glucose: C, 48·64; H, 8·11; OCH_3, 41·9.

A mixture with an authentic specimen of 2 : 3 : 6 trimethyl glucose began to melt at 85° C., and was all melted at 100° C. The two substances were clearly not identical. Furthermore, the crystals in 2 per cent. methyl alcoholic hydrogen chloride showed no inversion of sign in the rotatory power. The initial value obtained was $+78·43°$, decreasing to $+68·5°$ in 4 days ($C = 0·9$). Standing for a further 7 days brought about no diminution in this value. A mixed melt with an authentic specimen of 2 : 4 : 6 trimethyl glucopyranose (M.P. 115°) gave 113°–114° C.

Summary.

1. Attempts made at extracting laminarin sulphate undecomposed from laminariae fronds with dilute oxalic acid gave laminarin which contained very little combined sulphate.

2. Laminarin was converted to a triacetate which on methylation was converted quantitatively to trimethyl laminarin.

3. Hydrolysis of trimethyl laminarin gave a crystalline trimethyl glucose which was identified as 2 : 4 : 6 trimethyl glucopyranose. Laminarin, therefore,

is linked from the aldehydic carbon of one glucose unit to the third carbon of the next unit.

4. Examination of its properties suggests that laminarin consists of a chain of β-glucopyranose units bent into a spiral form.

The author wishes to express his gratitude to Professor T. Dillon for valuable advice given in the course of this investigation, and to Professor W. N. Haworth, F.R.S., for specimens of 2 : 3 : 6 and 2 : 4 : 6 trimethyl glucopyranose which he very kindly provided. Thanks are also due to the Royal Dublin Society for the loan of a polarimeter. Finally, grateful acknowledgment is made to the Industrial Research Council of Ireland, under whose auspices this work was carried out.

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No. 7.

A STUDY OF THE SEASONAL DEVELOPMENT OF THE POTATO PLANT IN RELATION TO BLIGHT ATTACK AND SPRAYING.

By PAUL A. MURPHY,
Albert Agricultural College, Glasnevin, Dublin.

[Read March 22, 1938. Published separately February 24, 1939.][1]

The present series of experiments had as one object to determine how long potato plants would continue growth and assimilation, and incidentally to discover how the development would proceed if there were no blight (*Phytophthora infestans*). A further underlying idea was the belief that differences between sprays could be measured more critically by their direct effects on the foliage than by their more indirect ones on the tubers. For this purpose it was sought to maintain one plot perfectly free from blight, another reasonably free as in commercial practice, and to leave the disease uncontrolled on a third, and then to determine the development of foliage and tubers on each by means of periodical weighings. It proved unexpectedly easy to eliminate blight, but more difficult to avoid injuring the plant in the process.

The experiment was begun in a provisional way in 1933, and was continued on an almost identical plan in 1934, 1936, and 1937, the three different treatments being compared on plots of 9/100 acre of Up-to-Date potatoes as follows:—

1. *Commercial Spraying.*—Three applications of Burgundy mixture containing 2 per cent. by weight of crystalline copper sulphate and 2·5 per cent. of crystalline sodium carbonate (except for the first two sprays in 1933 and 1934, which contained 1 per cent. and 1·25 per cent. respectively) applied on June 17, July 7, and July 31, 1933; June 22, July 14, and August 10, 1934; July 1, July 15, and August 5, 1936; June 30, July 16, and August 9, 1937.

[1] An account of this work was communicated to the Society by Prof. Murphy shortly before his last illness. Although he had virtually completed the paper, it was not quite ready for publication, and this was of necessity delayed until after his death.

2. *Weekly Spraying.*—Fourteen applications of a weak fungicide (the material varying in the different years) at average intervals of just over 7 days spread over a period of 95 to 98 days from June 22 to September 28, 1934, July 3 to October 6, 1936, and June 18 to September 21, 1937. In 1933 there were eight applications at intervals of 10½ days from June 17 to August 29.

3. *Control.*—No spraying.

Further details of the various sprays and the amount of copper applied per acre are given in Table 2. Each plot was walked through every week in 1937 to neutralise any mechanical harm done during the operation of weekly spraying.

On each of these areas the weights of haulms (stalks and leaves), leaves, and tubers were determined at intervals which averaged 10 days in 1933 and 1934, and 14 days in 1936 and 1937. This was done on four drills having a combined area of 1/100 acre (1/55 acre in 1937), which were so distributed over the plot that each was an "outside" drill during the last period before digging. The haulms and leaves were determined for 100 plants (20 in 1933; 52 in 1934) and are uniformly shown for 100 plants in the diagrams.

Although the amount of green foliage would have been the best measure of assimilating activity, it proved impracticable to separate the dead portions of leaves from the living, except on a minute scale, and a simpler and at the same time sufficiently accurate method was found by weighing the haulms (*i.e.*, the stalks and leaves together) in bulk on a dry day. Although the weight did not drop to zero when assimilation ceased, it was easy to make allowance for this, and the gross weight proved a good measure of assimilation. Actually the lower yellowing leaves soon dropped off, and those which died of blight shrivelled up in dry weather so that their weight became negligible. The total weight of compound leaves was determined at the same time; but although this reflected the effects of blight more promptly and approached nearer to zero at the end, its superiority was not great and did not compensate for the considerable labour involved.

The results are given with special reference to the 1937 experiment, which, profiting by previous experience, was on a larger scale and more uniform, and which shows the early stages of growth from June 21 onwards, as well as the later. The weekly spraying practised in that year was also nearly free from any appreciable checking effect on the foliage, which had caused some complication in the previous years, as referred to later.

Development and decline of Haulms and Foliage.

Neglecting the preliminary period of sprouting, the seasonal growth of the potato plant in Ireland may be said to cover six months from the time of

planting about April 1 to September 30. An analysis of this shows that it consists of four parts, as follows:—

1. From the inception of growth in the soil to the beginning of tuber formation, or from April 1 to June 16 (11 weeks) on the average, during which only roots and haulms are produced. The plants come above ground about May 1.
2. From the beginning of tuberisation to the apex of haulm production, which may be reached from July 24 to August 10, the average being August 5 (7 weeks), during which both haulms and tubers are increasing at the maximum rate. The haulms begin to meet across the drills near the end of June and blossoming begins about the same time and reaches its maximum about July 10–15.
3. A short period of about two weeks to August 19 on the average (varying from August 1 to 29) when there is no net increase in the weight of haulms, and crop production continues at a somewhat reduced rate.
4. A period of six weeks on the average from August 19 to September 30, during which both haulms and crop production decrease at a gradually accelerating rate.

This analysis refers to commercially sprayed crops. The course of events is much influenced by the blight attack (*Phytophthora infestans*), particularly in the last two periods, and the extent to which this is controlled by spraying.

Considering first the ideal case in which blight was all but perfectly controlled in 1937 by a treatment which caused no injury to the plants, the curve representing total weight of haulms (Fig. 14) forms an almost regular arc, rising to a maximum which was maintained from August 10 to 26, and then falling, slowly at first and afterwards more rapidly. The plot commercially sprayed with Burgundy mixture shows a curve of similar type, and the two follow an identical course up to about August 10, but they then diverge subsequent to the appearance of the first blight in the Burgundy plot at mid-August, with the result that the apex of the Burgundy curve is lower and occurs sixteen days earlier. This was due mainly to less perfect blight control on this plot, as was repeatedly noted from August 20 onwards. The blight attack, however, was not severe, and the latter parts of the two curves, which resemble each other and differ in shape from that of the control plot, represent in part death of the foliage from blight, but mainly a natural seasonal maturation which was advanced by the dry summer, and which brought growth on both plots to an end at the same time. The haulms of sprayed plots, which in the other years died from blight in the end, show a curve of different shape (see Figs. 12 and 13 for 1934 and 1936), which is like that of unsprayed plots, but occurs later. This was the case in 1934 and 1936, when the decline of the haulms of the two sprayed plots followed a sigmoid course about fourteen days and twenty-four days, respectively, later than the control.

This type of foliage decline was characteristic of the unsprayed plots in all years (least so in the drought of 1933), and is characteristic of the death of foliage from blight. There was an average attack of the disease in 1937, beginning on July 24 (compared with August 10, 1934, and July 3, 1936), and this period (July 27, 1937) marks the apex of the curve. A generalised diary of subsequent events in the three years is as follows:—

Haulms.	*Blight in field.*
Decline begins.	First blight spot noticed.
Very slow decline over 2 to 3 weeks, amounting to about 3 per cent. of maximum weight.	Foliage slightly spotted all over, but not visible at a little distance.
Rapid almost lineal decline over a period of 30 to 50 days, until the weight of the haulms is reduced to 15 per cent. of the maximum weight.	Early in period the foliage shows serious spotting on close examination, but still looks green. Midway in the period the plot looks grey-green in colour, when all the leaves are destroyed except tufts at the top of the stalks. A little later the plot looks brown, and just before this time the total weight of haulms is reduced to about 55 per cent. of their maximum, and simultaneously crop production ceases.
Slow decline during remainder of season.	Foliage almost completely dead and stalks beginning to whiten.

The production of tubers ceases abruptly when the haulms are reduced to about 55 per cent. of their maximum weight. At this time the weight of leaves is reduced to 30 to 40 per cent. of the maximum, the leaves naturally suffering more immediately from the attack than the stalks. Hence it was found in 1937 that measured by the weight of leaves the two sprayed plots showed the greatest superiority to the control on August 26, but measured by the haulms the date would be September 14. The same thing was noted in 1936, the corresponding dates being August 7 and September 2; but in 1934 the maximum difference for both leaves and stalks occurred on the same day, September 28.

The foliage of the control plot generally looked more luxuriant during the period following the beginning of spraying until the appearance of blight, particularly in 1934, when the outbreak was late. The haulms were somewhat taller, and the plot more difficult to walk through in consequence in July, 1934, and they continued making new growth longer, retaining their superiority until the end of August.

The differences just noted were not of great magnitude and needed close observation. The most delicate and obvious test of spray injury consisted of a

reduction and retardation of blossoming, which was obvious in 1934 and 1937 (no observation in the other years), as the following counts show:—

TABLE 1.

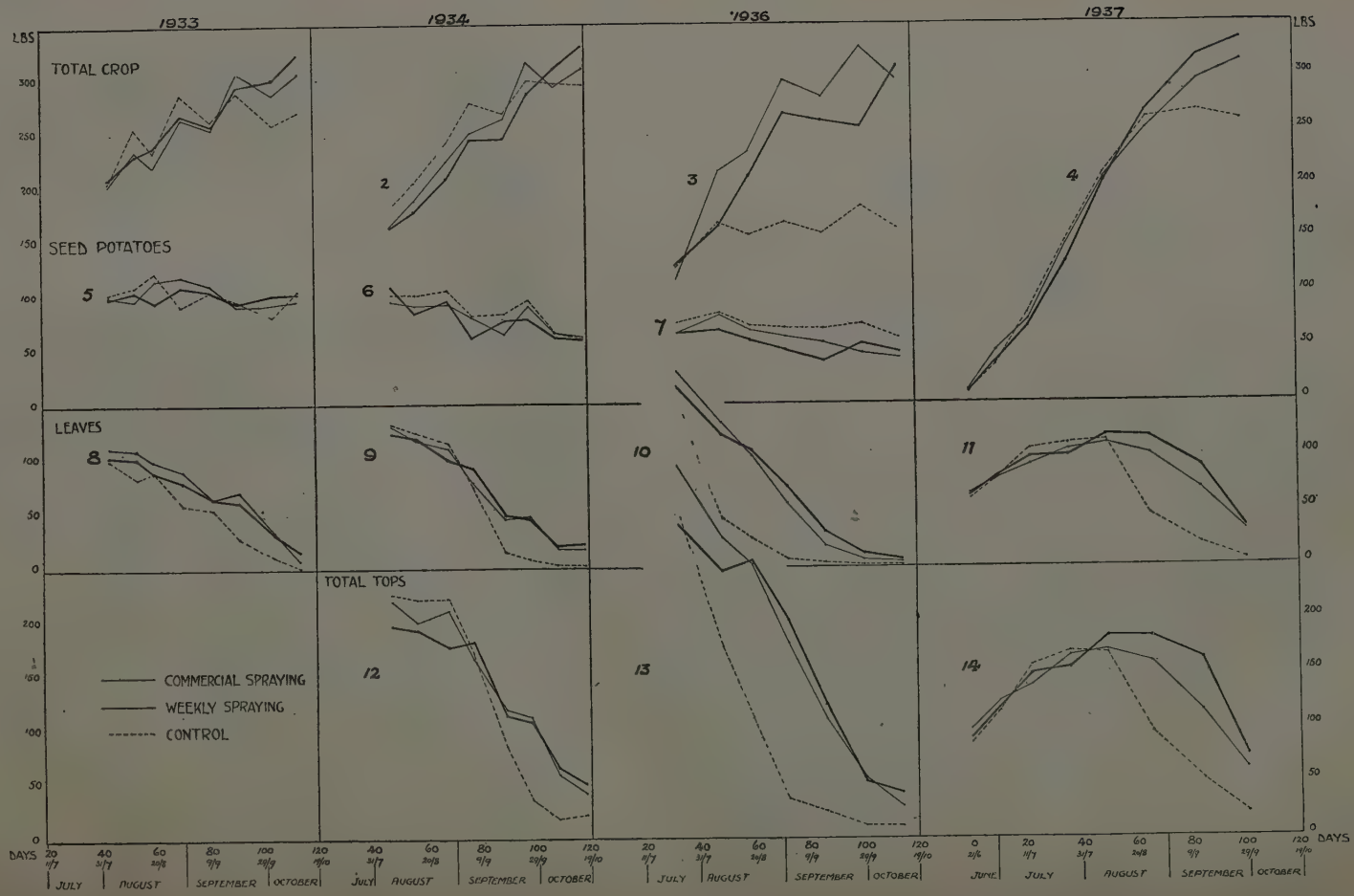
Year.	Treatment.	Effect on foliage.	Blossom Counts. 1st.	2nd.	3rd.
1934 ...	Control.	—	100	100	—
„ ...	Weekly Spray.	Checked.	77	37	—
„ ...	Burgundy.	Checked.	38	62	—
1937 ...	Control.	—	100	100	100
„ ...	Weekly Spray.	No check.	102	101	113
„ ...	Burgundy.	Checked.	76	85	98

The reduction on the Burgundy plot was visible from a distance in both years, and consisted mainly of a drop of the unopened blossoms, sometimes followed by increased flowering later, as in 1937, when the period was drawn out from the end of June to near the end of August.

The materials, strengths, and quantities used for the weekly spraying differed in the various years, as shown in Table 2, due to the development of injury in 1933, 1934, and 1936, and the desire to find a treatment which would be entirely harmless. This was finally attained in 1937 by the use of cuprous oxide, a weaker concentration, and small quantities of spray.

TABLE 2.

Year.	Material.	Copper content p.c.	Lb. per 100 gallons.	No. of applications.	Total gallons applied per ac.	Copper content of spray p.c.	Total copper applied per acre lb.
1933.	Bouisol ...	15	7½	8	1463	0·1125	16½
	Burgundy 1%	25	10	2	363	0·25	19
	„ 2%	25	20	1	198	0·5	19
1934.	Bouisol ...	15	5	14	1750	0·075	13
	Burgundy 1%	25	10	2	363	0·25	18
	„ 2%	25	20	1	181	0·5	18
1936.	Soltosan ...	51	2½	14	1980	0·1275	25
	Burgundy 2%	25	20	3	423	0·5	21
1937.	Cuprous oxide powder ...	55	0·9	2	214	0·05	5½
	do. ...	55	0·9	12	597	0·075	5½
	Burgundy 2%	25	20	3	263	0·5	13


1933
1934
1936
1937
LBS
TOTAL CROP
SEED POTATOES
LEAVES
TOTAL TOPS
2
3
4
5
6
7
8
9
10
11
12
13
14
COMMERCIAL SPRAYING
WEEKLY SPRAYING
CONTROL
DAYS
JULY
AUGUST
SEPTEMBER
OCTOBER
JUNE

The weekly spraying, while causing no direct injury as such, reduced the growth of both leaves and haulms in 1933, 1934, and 1936, so that they weighed consistently less than the Burgundy plots until the balance was redressed by blight in late August or September. The injury was not proportional to the amount of copper used, and appeared to be greatest in 1934, so that the form in which the copper was applied was a factor, although the conditions varied too much to allow of a direct comparison of Bouisol, Soltosan, and cuprous oxide. The number of applications was another factor, for continued applications of weak Bouisol and Soltosan had a continuous depressing effect on growth which must be attributed to cumulative copper poisoning. This was probably proportional in each case to the total amount of copper applied per acre. The experiment provides no direct indication as to whether the excess copper was more harmful at one period than another, but the evidence indicates that all the copper fungicides depressed the growth of foliage and tubers at first, except in the very dry year of 1933, when the foliage was increased throughout the season and the crop reduced at first.

Weak cuprous oxide (used for the first two applications at 0·05 per cent., and for the remaining twelve at 0·075 per cent.) caused no appreciable injury, and at the same time gave perfect control of blight to the end of the season. The quantity of copper in this case was designedly cut down and amounted only to 5½ pounds per acre per season, that is about one-half of the copper content (12 lb.) of three applications of Burgundy mixture (1 per cent., 1 per cent., and 2 per cent.) at 120 gallons per acre, which give less complete control. Evidently the amount of copper used in commercial spraying is excessive, and at the same time is not used to the best advantage. Such a treatment might be included in spraying experiments instead of an unsprayed plot where it is desired to have a standard against which to measure various spraying treatments, without the complications from "neighbour effect" which an unsprayed plot introduces. This question is discussed later.

This undoubted check to growth was also reflected in the crop in the middle portion of 1933, 1934, and 1937, with the result that the control plot consistently showed the highest crop yield during this period, just as it showed the most leaves and haulms. In addition the harmful repeated applications were generally in the lowest place in all three respects (1934 and 1936). At the same time the increase in the crop was hardly in proportion to the greater luxuriance of the unsprayed potatoes in July and August, and it is clear that the crop is not always in direct proportion to the haulms, a fact well known to potato growers. The decline of the foliage was more particularly observed in 1937, on the plot sprayed weekly, and the matter is worth considering at some length, as it has not apparently been studied previously. It is not merely that growth of new leaves ceased on August 26, and that the old ones gradually died subsequently. Actually the lowest leaves began to yellow-off on July 13, probably due to shading, and this continued at an increasing rate from late July onwards, and especially during September. At the beginning of that

month the plants had taken on a distinct autumnal appearance, due to the recumbent position of the stalks, yellowing or bronzing of the foliage, and dropping of the lower leaves. Examination of a typical plant on September 28 showed the following condition :—

Main stem to bifurcation.—All 11 leaves from the ground up fallen. Axillary shoots slightly developed, but none of their leaves expanded.

Right branch.—Four lower leaves green and for most part alive. Six upper leaves dead, or practically so, and hanging.

Left branch.—Four intermediate leaves for most part green. Eight remaining leaves dead and hanging.

These plants had been producing new foliage at the top continuously during the greater part of September, and the amount of living foliage at any time from mid-July onwards was, therefore, not static but represented a balance between loss below and gain above. The last leaves to be produced were very thin and light-green in colour, and finally the plants tended to die from their two extremities, the large lower leaves dropping, and the thin top leaves yellowing all over or turning brown at the edges, and gradually dying from the tip of the leaflet backwards, and remaining hanging.

It must be emphasised that blight had almost nothing to do with this process. The decline of plots dying from the disease shows a different curve, and the plants themselves present a different picture, for the terminal leaves are generally the last to go. The death of the plants must be considered a normal seasonal maturity, due to the individuality of the variety, the weather, and possibly decreasing light. The dry summer certainly hastened maturity, for although the plants did not actually die from drought, they would have lived longer in a wetter season. Again, although the variety (Up-to-Date) is a main-crop one, there are others which grow on longer. Still it is clear that even in the absence of blight most main-crop potatoes reach a natural maturity from about the end of September to about October 20, depending on the year and variety, and it is impossible to keep them alive any longer.

Development of the Tubers.

The course of tuber production is best studied in the case of the repeatedly sprayed plot in 1937, which represents perfect blight control without any evidence of phytocidal effect.

The curve for total crop has a sigmoid shape, being determined by two factors, the rate of assimilation and the destination of the food produced. As will be seen from Fig. 4 the rate of tuber production from June 21 to mid-July was slower than from mid-July to mid-August. This was in part due to a smaller initial amount of foliage, and in part to the fact that the development of the haulms was the reverse of that of the tubers, being more rapid at first

and then slower, since more of the assimilated food went into the leaf apparatus than the tubers during the early part of the season.

The periodical diggings were made at approximately fortnightly intervals, and it is simple to calculate the yields for regular fortnightly periods, seven in number, extending from June 21 to September 27. This date was three days before the last digging, but there are reasons to believe, as shown below, that production had almost ceased at that time. The results are shown in Table 3, from which it will be seen that tuber production was at a maximum which remained practically constant over a period of four weeks from July 19 to August 16, the increase per week being 1½ tons per acre. This period also coincided approximately with the peak of the foliage curve. Soon afterwards the net amount of foliage began to decline from natural causes, and a little before this tuber production also slowed down gradually, at first apparently as a direct result of the shortening days, and afterwards due to the rapid decline of the haulms in addition.

On the commercially sprayed plot the maximum tuber production was at the rate of 1½ tons per week also, but it occurred earlier, and was maintained over one fortnightly period only, from July 19 to August 2. Corresponding with this the peak of the foliage curve was also advanced from August 10 to July 27 after which it fell away more rapidly. The consistently higher yield of the repeatedly sprayed plot in the latter part of the season is ascribable to this decline. Crop production in the control plot showed a slight superiority over both the sprayed plots in the middle portion of the season of 1937 (July 8 to August 18), which in itself might not be significant were not the same the case in 1934 (August 5 to September 14). These were not merely coincidences, but were related to more vigorous haulms, at least in 1934 and 1936, and they indicate somewhat greater assimilation and crop production between the beginning of spraying and the appearance of blight, due to the absence of phytocidal action. The maximum production of almost 1½ tons per week was reached on July 19, and lasted for two weeks until August 2. After the blight became general on August 6, the rate of production began to decline, and ceased abruptly on August 26. The effect on the crop of the phytocidal action exerted by the repeated spraying practised in 1934 and 1936 is clearly shown by the reduced yield shown by these plots compared with Burgundy mixture during the latter part of the season (Figs. 2 and 3).

As already explained, the method of weighing the stalks in bulk did not allow a direct estimation of the amount of functioning foliage remaining at any time. Thus when the control had about ceased production on August 26, 1937, the haulms still weighed 53·5 per cent. of their maximum on July 27; but at that time there were few green leaves left, and the bulk was made up mainly of the bare but still green stalks, and to a less extent of dead adhering leaves. It is probably a legitimate general conclusion that when the weight of haulms has been reduced to about 55 per cent. of the maximum, tuber production has about ceased. At the same time the decline in the weight of leaves, which

is very rapid, has been reduced to a figure between 30 and 40 per cent. of the maximum. At this stage the field looks grey-green at a distance, the stalks are still green, but all the leaves are dead, except for a tuft of six, or so, more or less green ones at the apex of the shoots.

It would appear that this theorem can also be applied with approximate accuracy to sprayed plots, and to a seasonal decline of the foliage in which blight plays little part. The weights of the haulms on the commercially sprayed and perfectly sprayed plots were reduced by 50 per cent. on September 24 and 26, respectively, and these had practically stopped tuber production at that time, for they were judged incapable of further assimilation on September 28.

It is a further corollary that the time interval between the stoppage of production on the control plot (August 28) and that on the two sprayed plots (average September 25), amounting to 28 days, was principally, but not entirely, responsible for the resulting difference in yield, the other factor being the more rapid decline of the control foliage previously. Not only is the length of this interval important, but also the time at which it occurs, for 28 days in August are worth nearly twice as much as 28 days in September (the production being 4·05 tons against 2·26 tons). The yield superiority of sprayed potatoes, therefore, depends on three factors which may be stated as follows:—The sooner unsprayed potatoes die, (1) the smaller will be their crop; (2) the longer is likely to be the interval before corresponding sprayed potatoes die; and (3) the earlier will that interval begin, while the rate of production is still high.

TABLE 3.

Total Crop production calculated for Fortnightly Periods, 1937.

Period.	Date.	Fortnightly increase in the total crop in Tons per acre.		
		Complete control.	Commercial control.	Unsprayed.
1	June 21–July 5 ...	1·74	2·08	1·85
2	July 5–July 19 ...	2·16	2·19	2·71
3	July 19–Aug. 2 ...	2·97	3·01	2·91
4	Aug. 2–Aug. 16 ...	3·03	2·36	2·49
5	Aug. 16–Aug. 30 ...	2·34	1·69	1·53
6	Aug. 30–Sept. 13 ...	1·69	1·52	0·22
7	Sept. 13–Sept. 27 ...	0·71	0·74	—
	Totals, ...	14·64	13·59	11·71

These facts are clear from Table 3, but it will be useful to give the actual monthly total production of the sprayed and unsprayed potatoes as follows:—

Treatment.	June.	July.	August.	September.
	Tons.	Tons.	Tons.	Tons.
Commercial Spraying ...	1·33	5·56	4·58	2·15
Weekly Spraying ...	1·12	5·32	5·91	2·29
Unsprayed	1·19	5·86	4·45	0·21

The high figure for July in the last case represents the absence of any phytocidal effect, especially as compared with Burgundy mixture. The variations in yield in June are probably due to variations in the time that otherwise uniform plants begin to set tubers.

The low production in September is remarkable, even when blight was completely controlled, indicating that there is a seasonal limiting factor here which cannot be obviated. Tuber production for that month in both the sprayed plots was not much more than that for June, which was the lowest month of any. Since the production figures for July and August also come near balancing each other, it is consequently found that about half the crop has been produced by the end of July. This period marks a general turning point for the crop, for the foliage then reaches almost to its greatest development and the rate of tuber production is at its maximum. The better blight is controlled, the later the half-way mark for crop production comes; in the present work it fell on August 3, July 30, and July 24, respectively, in the complete control, commercial control, and unsprayed plots.

Blight appeared later in 1937, and the benefit from spraying practically consisted of the extra crop produced in September, and was therefore of moderate dimensions. In districts in which the blight normally appears later than this, the benefit would be still less, and this consideration probably accounts for the usual neglect of spraying under these conditions. Thus if unsprayed potatoes remain alive until the end of August, spraying produces an increase of 2¼ tons, but if they survive until about the middle of September the increase is only ¾ ton. Furthermore, under such conditions the normal maturity would tend to be advanced, which still further reduces the benefit. It takes an increase of about ½ ton of potatoes to pay for two applications of 1 per cent. Burgundy mixture (estimated to cost 25 shillings), and allowing a margin for the necessary profit on the operation, spraying is only likely to justify itself where it results in extending the productive period by three weeks in September, thereby increasing the crop by about 1½ tons per acre. It appears to be useless to endeavour to keep the crop growing later than the end of September by spraying or other means, apart from the added danger of blight infection in the tubers which would follow.

The rate at which medium-sized tubers suitable for seed purposes (between 2″ and 1¼″) are produced is a matter of importance. Unlike the curves for total crop and marketable crop, which rise continuously until the end of the season, that for seed potatoes remains almost constant with a slight drop, particularly in September. Evidently in the last stage of growth the tubers increase more in size than in number. The average production of total crop and of seed potatoes for the four years, 1933, 1934, 1936, and 1937, is shown in Figs. 1–7. The curves were of the same type in each of the years, but the weight of the seed crop was highest in the dry season of 1933.

Discussion.

When the present work was begun in 1933 the only known work bearing on the rate of increase consisted of early observations of Jones (6), that 321 bushels out of a total crop of 379 bushels were produced in the last 51 days of a growth period of 125 days. Since then considerable work on the present lines has been reported from the United States.

Blodgett, Mader, and their co-workers at Cornell, found, as announced in a series of papers extending from 1933 to 1937 (2, 8, 9, 10, 11, 12), that spraying with Bordeaux mixture in Western New York generally increased the weight of foliage and the final weight of the crop, but reduced the crop in the early part of the season. A similar initial crop reduction was found in other experiments by Bonde (4) in Maine, where it was afterwards made good, and by MacLeod and Dickison (7) in Long Island, where it generally was not. In addition, Mader and Mader (12) observed in 1937 that spraying also retarded and reduced blossoming.

The similarity to the present results is clear, and evidently such phenomena as retarded blossoming and initial reduction of the crop following the application of copper fungicides are of wide occurrence in spite of widely different climatic and parasitological conditions. In New York spraying increased the weight of foliage, partly by controlling insects, but apparently principally by making the plants better able to withstand unfavourable conditions, mainly drought, or by an actual stimulative action (9), but the evidence indicates that under ordinary conditions in Ireland any copper spray is directly harmful to some extent at first, or at best neutral, and only becomes beneficial when it reduces the blight attack later on. It is unlikely, therefore, that the potato plant will be found to withstand a stronger spray in the early part of the season in this country, or to be benefited by it, as has been found to be the case in the United States (8). It was remarkable, however, that in the very dry season of 1933 both spray treatments increased the weight of foliage but reduced the crop at first, thus confirming in all respects the results of Mader, Blodgett, and their co-workers (9, 10, 11, 12), which were obtained under similar conditions, that is, in a dry

climate and dry seasons, when blight was of no consequence. This would appear to be mainly a climatic effect, because insects were no factor in that or any other year at Glasnevin.

The amount of copper applied per acre per season in New York (75 lb. of copper sulphate, in the case of the Rural variety, approximately equivalent to 19 lb. of copper) was much above the normal complement (about 12 lb. of copper) used in Ireland, and it is apparent that the potato plant is more sensitive to copper poisoning in most years here than in America, and that the injury is most severe in wet climates and seasons, just as in the case of apples (5). The different varieties grown in the two countries may also be a factor, since the optimum amount for the Green Mountain potato was found to be only 30–40 lb. of copper sulphate under the same conditions in New York (9).

SUMMARY.

The rate of growth of foliage and tubers was studied on potatoes of the variety Up-to-Date on which blight (*Phytophthora infestans*) was perfectly controlled, commercially controlled, and uncontrolled.

The first effect of spraying is generally to check the production of foliage and tubers and reduce blossoming, so that spraying becomes beneficial only when it reduces the blight attack later on. In the abnormally dry year of 1933, however, spraying increased the foliage, as has been found under similar conditions in the United States.

Perfect control of the disease without appreciable injury to the plants was obtained by weekly applications of a dilute solution of cuprous oxide, the total amount of copper applied to the crop during the season being less than half that applied in ordinary commercial spraying with Burgundy mixture.

In the blight-free crop maximum production reached 1½ tons per week, which was kept up for four weeks following blossoming, after which it decreased, so that the September production was little more than one half that of August. The period of maximum tuber production coincided approximately with that of maximum foliage production, and the curve for total weight of haulms forms an almost regular arc.

Following blight attack on the unsprayed crop, the decline in the production of haulms takes a sigmoid course; production of tubers ceases abruptly when the haulms are reduced to 55 per cent. of their maximum weight, the leaves at this time being 30 to 40 per cent. of their maximum weight.

The extent of the benefit of spraying on production depends on the interval between the stoppage of production due to blight on the foliage and that due to normal maturation about the end of September.

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No. 8.

ATMOSPHERIC POLLUTION IN DUBLIN DURING THE YEAR 1938.

By

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A MOVEMENT for the systematic investigation of atmospheric pollution arose from a conference of delegates from municipal authorities and others held in connection with the Smoke Abatement Exhibition in London in 1912. A committee was appointed, under the chairmanship of Sir Napier Shaw, of the Meteorological Office, "to draw up details of a standard apparatus for the measurement of soot and dust." The reports of the committee were originally published in the *Lancet*; but as the work increased, owing to a greater number of municipal authorities taking part, the coordination of results was transferred to the Department of Scientific and Industrial Research in 1927, the reports from that time being published annually by H.M. Stationery Office.

There is now available a large collection of data in the reports of the Atmospheric Pollution Research Committee as the result of systematic observations extending over many years. In Great Britain during 1937 there were 87 co-operating municipal and industrial bodies maintaining observations at 133 centres. The apparatus and methods of analysis have been standardized by the Atmospheric Pollution Research Committee, so that results at different centres are readily comparable; further, it may be remarked that the experimental methods are simple, and present no serious difficulty to anyone trained in methods of chemical analysis.

Measurement of Deposited Solids.

Rain-water is caught in a glass funnel of one foot diameter and is collected in a ten-litre bottle attached to the funnel. Each funnel is tested at the Fuel Research Station, and factors sent out with the gauge whereby the weights of deposits found may be converted into metric tons per square kilometre or English tons per square mile.

The funnel and bottle are placed in the open, away from buildings, and at the completion of each monthly period any solid matter remaining in the funnel is rinsed out with some of the collected rain-water which is returned to the collecting bottle. The quantity of water is determined by weighing.

After standing in the bottle for some days, the supernatant liquid is siphoned off, and the quantity of insoluble solids determined by filtration through a Gooch crucible and drying at 100° C. Extraction of the solids by carbon disulphide gives the amount of "tarry matter," while ignition of the residual matter in the crucible causes a loss in weight due to "carbonaceous matter other than tar," the final residue in the crucible being "ash."

The soluble solids are estimated by evaporation of 1 or 2 litres of rain water in a weighed silica dish, and drying at 100° C. Gentle ignition of the solids gives the "loss on ignition," while the residue constitutes "ash."

1 or 2 litres of water are evaporated to low bulk and the sulphate present precipitated as barium sulphate, the weight of which gives a measure of "SO_3" in solution.

Chlorine, present as chloride, and ammonia are determined by silver nitrate solution and Nessler's reagent, respectively, as in water analysis.

Observations at Leinster Lawn, 1938.

The results in the following table cover the period from 6th January to 31st December, 1938, and are expressed as metric tons (1000 kilograms) per 100 square kilometres, this being the practice followed in the reports of the Atmospheric Pollution Research Committee. Division by 40 will convert the figures very nearly into English tons per square mile.

The letters A and B in the fourth line from the bottom of the table refer to the classification of cities, adopted by the Atmospheric Pollution Research Committee, according to the degree of pollution by the constituent concerned.

DEPOSIT TABLE.

Impurity Deposited from the Atmosphere at Leinster Lawn from January 6th to December 31st, 1938.

—	Rain-fall.	Grams per square Dekameter (Metric Tons per Hundred Square Kilometres).								
		Insoluble Matter.			Soluble Matter.		Total Solids.	Included in Soluble Matter.		
	mm.	Tar.	Carbon-aceous other than tar.	Ash.	Loss on Ignition.	Ash.		Sulphates (SO_3).	Chlorine (Cl).	Ammonia (NH_3).
January (25 days) .	68	14	99	253	198	207	771	70	139	10
February . . .	12	10	112	259	76	135	592	42	39	4
March . . .	19	9	110	243	57	140	560	56	18	4
April	2	7	134	273	23	130	567	40	11	2
May	59	7	163	212	85	114	580	52	18	4
June	77	8	102	212	80	115	517	46	27	6
July	100	7	103	141	49	133	435	53	40	2
August . . .	56	8	112	167	84	123	494	50	20	5
September . . .	33	7	82	141	23	104	357	36	17	2
October . . .	106	11	125	211	164	254	765	93	132	3
November . . .	72	14	86	193	89	140	522	47	72	3
December . . .	93	9	105	162	65	149	491	63	75	3
Mean Monthly . .	58	9 B	111 B	205 B	83 B	145 A	554 B	54 A	51 B	4 A
Summer Total . .	327	44	696	1,146	344	719	2,948	277	133	21
Winter Total . .	370	67	637	1,321	649	1,025	3,701	371	475	27
Annual Total . .	697	111	1,333	2,467	993	1,744	6,649	648	608	48

The classification is based on the mean monthly figures of pollution and is as follows:—

	A	B		C		D
	Less than	Equal to or more than	Under	Equal to or more than	Under	Equal to or more than
Insoluble Matter						
Tar	5	5	15	15	25	25
Carbonaceous other than tar ...	100	100	300	300	500	500
Ash	200	200	600	600	1,000	1,000
Soluble Matter						
Loss on ignition ...	75	75	225	225	375	375
Ash	150	150	400	400	750	750
Total Solids ...	500	500	1,500	1,500	2,500	2,500
Sulphates (SO_3) ...	100	100	300	300	500	500
Chlorine (Cl) ...	30	30	90	90	150	150
Ammonia (NH_3) ...	5	5	15	15	25	25

From the deposit table it will be seen that from the point of view of soluble ash, sulphates, and ammonia, Leinster Lawn falls into the A class, but on the remaining six constituents it goes into class B. The most striking seasonal variation is shown by chlorine, the winter total being more than 3·5 times that for the summer. Such marked variation is common at towns close to the sea coast.

Sulphates and tar in winter are noticeably higher than in summer, a result which is to be ascribed to greater coal consumption in the winter months. The effect of rainfall on certain constituents is noticeable; thus insoluble matter was a maximum in April when the rainfall was a minimum, and soluble matter was a maximum in October when the rainfall was a maximum.

A comparison with results in 1936–37 in other towns may be of interest.

Annual Deposits in Metric Tons per 100 *square kilometres.*

	Rainfall in mm.	Tar.	Total Solids.	SO_3	Cl.
Cardiff	1,187	52	7,174	863	739
Coventry (City Hospital)	803	77	6,567	960	319
Dublin (Leinster Lawn)	697	111	6,649	648	608

Annual Deposits in Metric Tons per 100 square kilometres—continued

	Rainfall in mm.	Tar.	Total Solids.	SO_3	Cl.
Glasgow (Bellahouston Park) ...	894	45	7,308	1,072	801
,, (Alexandra Park)	895	169	13,621	1,581	1,022
,, (Botanic Gardens)	904	99	8,984	1,149	564
Liverpool (St. George's Hall) ...	642	183	14,534	1,182	2,322
London (Battersea Park)	727	220	9,929	1,275	452
,, (Finsbury Park)	699	181	10,915	2,136	463
,, (Golden Lane)	623	288	10,453	1,544	897
,, (Sth. Kensington)	698	194	8,776	1,088	530
,, (Kew Observatory N.) ...	714	91	4,674	666	300
,, (,, ,, S.) ...	739	92	5,100	796	331
Manchester (Booth Hall)	927	117	7,346	1,066	1,361
,, (Oldham Road) ...	956	500	21,088	1,933	1,568
,, (Phillip's Park) ...	975	242	20,223	1,691	1,733
Southport (Hesketh Park)	992	45	4,580	555	1,138
Widnes (Isolation Hospital) ...	849	54	9,938	1,419	1,329

The first four stations on the above list show total solids of about the same value; the tar deposited in Dublin is, however, much the highest, while the sulphate is the lowest. The low figure for sulphate is borne out by independent measurements made by the lead peroxide method, to be described later. The high figure for tar may possibly be accounted for by the high ratio of domestic to industrial smoke in Dublin, domestic soot containing a much higher content of tar than industrial soot, or possibly to differences in the quality of coals used here and elsewhere. The high figures for chlorine in the last column correspond to towns situated near the west coast of Great Britain, and are largely to be accounted for by salt-laden winds from the Irish Sea.

SUSPENDED IMPURITY.

In addition to matter which settles from the air, there is also suspended matter which does not settle owing to its extreme state of division. Suspended impurity passes into the lungs when we breathe, and is of direct importance with reference to health. An automatic filter was devised by Owens (Rep. IV, A.P.R.C.) whereby 2 litres of air are drawn through filter paper rotated by clock-work at regulated time intervals, leaving a dark spot 3 mm. in diameter, the depth of shade of which can be matched by reference to a set of standard

shades. The unit of shade corresponds to 0·32 milligrams of suspended matter per cubic metre.

The instrument used in Dublin was set up on the north side of University College, Upper Merrion Street, and is actually situated about 70 yards from

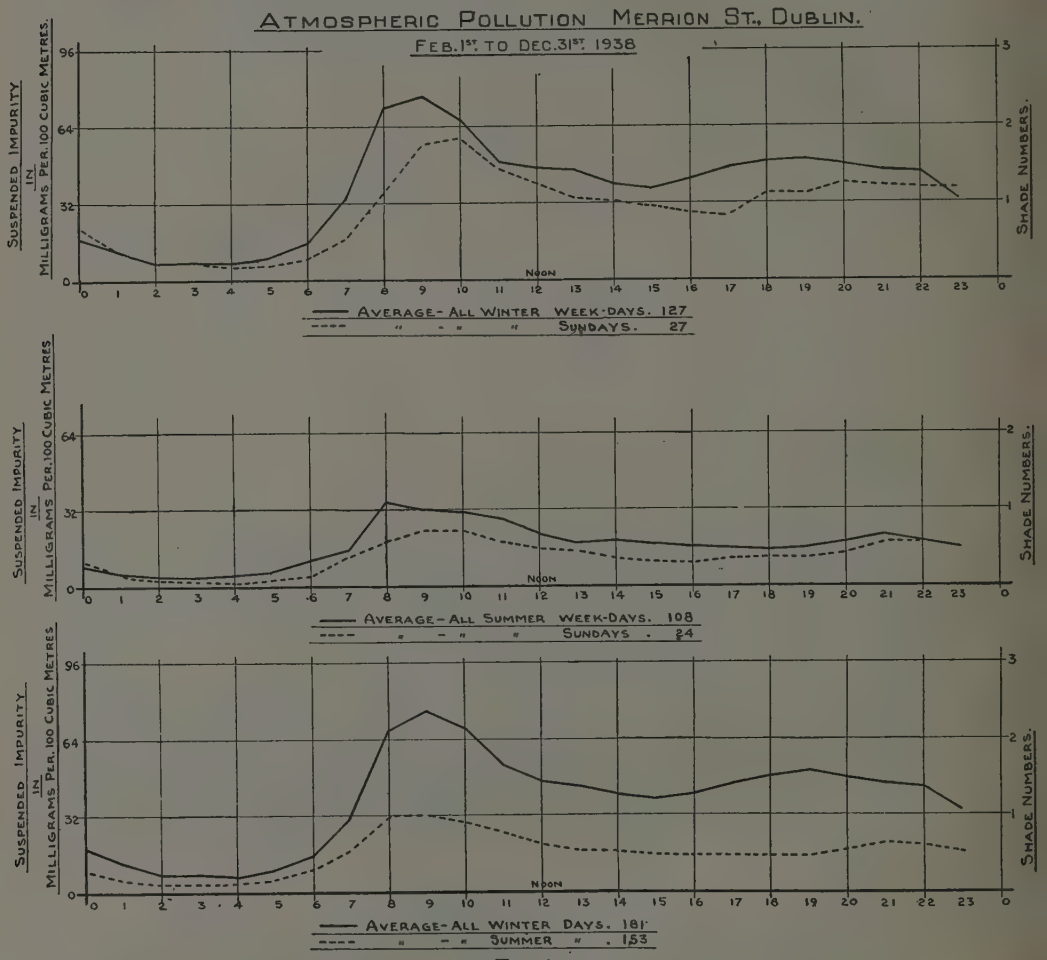
ATMOSPHERIC POLLUTION MERRION ST., DUBLIN.
FEB.1ST. TO DEC.31ST. 1938
SUSPENDED IMPURITY IN MILLIGRAMS PER.100 CUBIC METRES.
SHADE NUMBERS.
NOON
AVERAGE-ALL WINTER WEEK-DAYS. 127
" - " " SUNDAYS. 27
AVERAGE-ALL SUMMER WEEK-DAYS. 108
" - " " SUNDAYS . 84
AVERAGE-ALL WINTER DAYS. 181
" - " SUMMER " . 153

FIG. 1.

the standard gauge used for deposited solids in Leinster Lawn. Work with the filter commenced on February 1st, 1938.

The normal trend of the daily graph for suspended impurity shows a minimum value in the early morning about 4 a.m., followed by an increase connected with the lighting of fires, reaching a peak about 9 or 10 a.m. The

value then falls off, but may rise slightly in the afternoon and evening, especially in the winter months.

Hazy and foggy days lead to high values of impurity and corresponding increased depth of shade on the filter paper. The Atmospheric Pollution Research Committee classify as "hazy" or "Z" all days upon which a shade depth of 4 units is reached at any time during the day. Such a shade value corresponds to 128 mg. suspended impurity per cubic metre.

Results at Upper Merrion Street, 1938.

In figure 1 the upper graphs give average values for all week-days, excluding Saturdays, and the average values for Sundays and holidays during the six months, February, March, April, October, November, and December. The curve for week-days lies almost entirely at a higher level than that for Sundays,

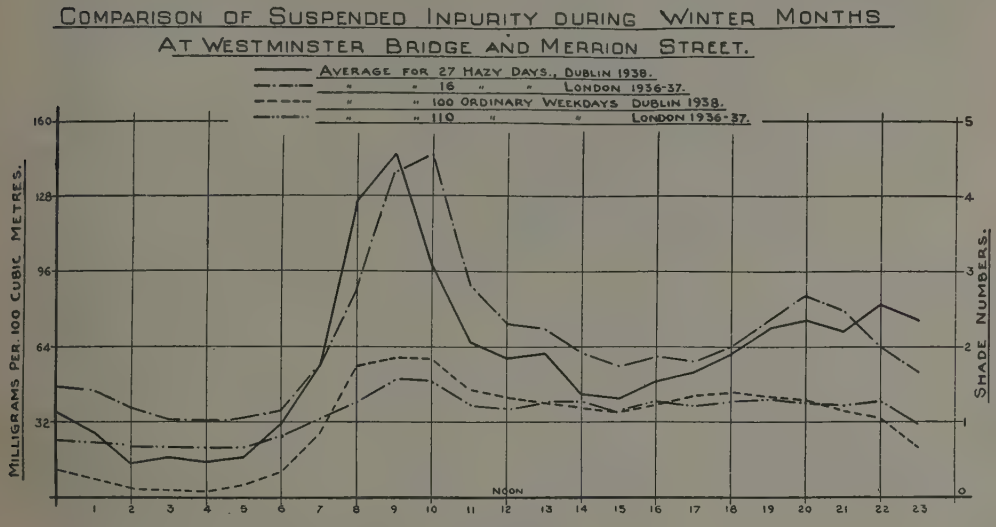
COMPARISON OF SUSPENDED IMPURITY DURING WINTER MONTHS
AT WESTMINSTER BRIDGE AND MERRION STREET.
AVERAGE FOR 27 HAZY DAYS., DUBLIN 1938.
" " 16 " " LONDON 1936-37.
" " 100 ORDINARY WEEKDAYS DUBLIN 1938.
" " 110 " " LONDON 1936-37.
MILLIGRAMS PER 100 CUBIC METRES.
SHADE NUMBERS.
160
128
96
64
32
5
4
3
2
1
0
NOON
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 2.

the difference between the two giving a rough approximation to the pollution due to industrial chimneys, on the assumption that the latter are idle on Sundays. A comparison of the areas gives the ratio of domestic to industrial pollution as 3·3 : 1.

The central graphs show the corresponding values for the months May, June, July, August, and September. The ratio for domestic to industrial pollution being 2·3 : 1.

The lower graphs give the average values on all winter and all summer

days. Comparison of the two areas shows the average daily winter pollution to be 2·4 times that of the summer.

The graphs in figure 2 give average results for "hazy" winter days and "ordinary" winter week-days (excluding Saturdays) compared with the corresponding days at Westminster, 1936–37.

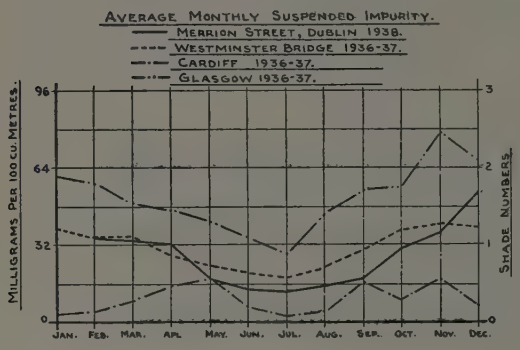

FIG. 3.

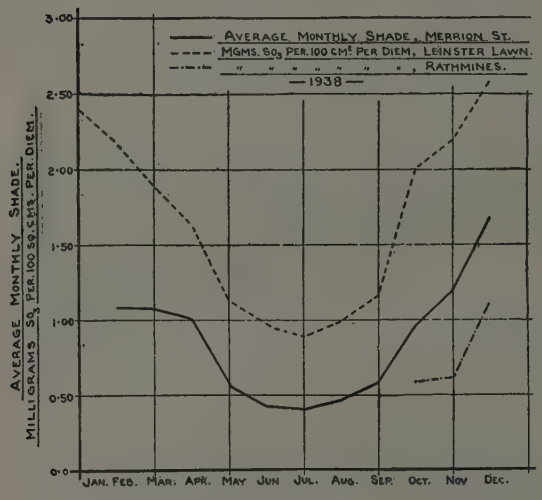
AVERAGE MONTHLY SHADE, MERRION ST.
MGMS. SO3 PER. 100 CM² PER DIEM, LEINSTER LAWN.
" " " " " " " , RATHMINES.
— 1938 —
AVERAGE MONTHLY SHADE.
MILLIGRAMS SO3 PER. 100 SQ. CMS. PER DIEM.
3·00
2·50
2·00
1·50
1·00
0·50
0·0
JAN. FEB. MAR. APR. MAY JUN JUL. AUG. SEP. OCT. NOV DEC.

FIG. 4.

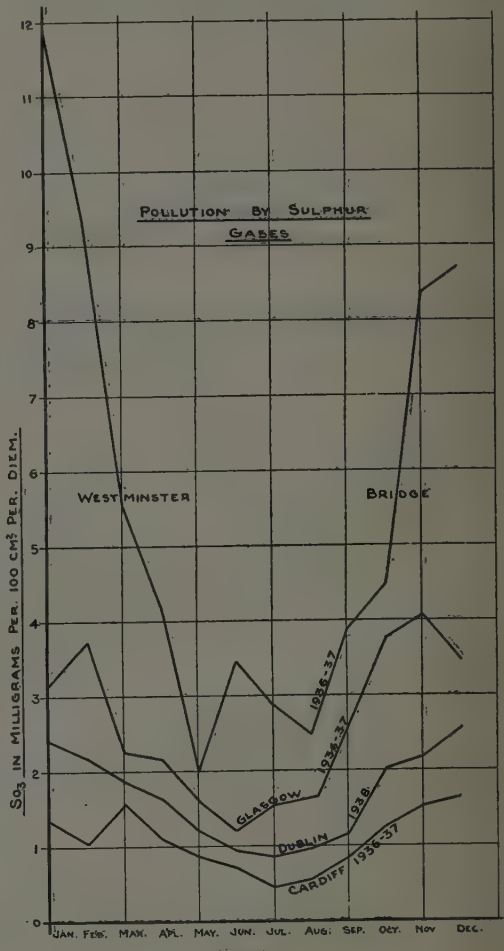

FIG. 5.

It will be seen that, except for the early morning hours from midnight to 7 a.m., the corresponding curves lie fairly close one to the other, and therefore the winter level of suspended impurity at Merrion Street approximated to that obtaining at Westminster Bridge.

By summing up all the shade numbers for every hour and day of the month and dividing by the total number of hours, an average shade number for the month is obtained. Figure 3 gives a graph of such shades for Dublin compared with those for Cardiff, Glasgow, and Westminster Bridge in 1936–37. The curves for Dublin and Westminster Bridge are fairly close in the winter months, but the level of pollution during the summer is considerably lower in Dublin. Glasgow shows a much heavier degree of pollution throughout the year, while the graph for Cardiff shows lower pollution with a peculiar distribution, giving minima in January, July, October, and December, and maxima in May, September, and November.

Effect of Wind Velocity and Direction on Suspended Impurity.

During the period February 1st to December 31st 40 hazy days, i.e., days upon which a shade number of 4 occurred, were recorded. From observations of wind velocity kindly supplied by Mr. J. Gilmour, Trinity College, it was found that such hazy days were also days of low wind velocity.

Observations of wind velocity are made at Trinity College each day about 9 a.m., which happens to be the time when pollution by suspended impurity is usually nearing its daily maximum. A summary of the shade numbers recorded by the automatic filter at 9 a.m. on winter days is given in the following table under the corresponding wind velocities (Beaufort Scale):—

The winds are classified to some extent according to direction.

Average Shades for Varying Wind Velocities.

Wind Direction.	Beaufort Numbers.								
	0	1	2	3	4	5	6	7	8
Calm	5·5 (11)	—	—	—	—	—	—	—	—
N.E., E.N.E.	—	4·0 (2)	—	0·5 (1)	—	—	—	—	—
E.	—	2·8 (5)	1·2 (3)	1·9 (5)	1·4 (5)	—	—	—	—
E,S.E., S.E., S.S.E.	—	2·0 (3)	2·2 (7)	1·3 (5)	1·2 (4)	1·2 (3)	—	—	—
S.S.W., S.W., W.S.W.	—	1·9 (7)	2·0 (7)	1·7 (21)	2·0 (3)	1·5 (2)	—	—	—
W.	—	4·0 (9)	2·5 (15)	1·8 (21)	1·2 (4)	—	—	—	1· (1)
W.N.W., N.W., N.N.W.	—	3·3 (7)	2·7 (10)	2·4 (7)	1·3 (3)	2·0 (1)	—	—	—

[Figures in brackets give the number of days from which the average is taken.]

The averages in several cases are taken from too few records to be reliable, but in general it is seen that high-shade numbers are only found when wind velocities are low. Winds from a westerly quarter appear to carry rather greater pollution than those from the eastern quadrant. It would be wiser, however, to accumulate more data before making any definite conclusion on this point.

Sulphur Dioxide.

Average bituminous coal contains about 1·5 per cent. of sulphur which when burnt gives rise to sulphur dioxide. City air always contains a small proportion of this gas which is harmful in its effect on building stone, particularly those of a sedimentary type. Two methods are used for measurement of sulphur dioxide, one volumetric and one gravimetric.

In the first method (Ellis, Rep. XVII, A.P.R.C., p. 40), a measured volume of air is bubbled through a neutral solution of hydrogen peroxide, and the sulphuric acid thereby produced is titrated with standard alkali. The method is simple, but daily determinations are necessary if continuous records are required. Results are expressed as parts of SO_2 per million volumes of air.

The second method, which is the one employed in Dublin, depends upon the absorption of sulphur dioxide by lead peroxide, thereby producing lead sulphate,

$$SO_2 + PbO_2 = PbSO_4.$$

A piece of cotton fabric of suitable size is wrapped round a special porcelain cylinder, and a paste of 8 g. of PbO_2 in a mucilage of gum tragacanth is applied to the fabric. The paste is then dried in a desiccator over quicklime. The surface of PbO_2 on the cylinder measures 100 sq. cm. A cylinder prepared as above and protected by a metal cowl is exposed to the atmosphere for one month.

The fabric and PbO_2 are then stripped off, boiled with a solution of 5 g. Na_2CO_3, whereby the insoluble $PbSO_4$ yields soluble Na_2SO_4, the solution of Na_2SO_4 is filtered off and after acidification with HCl treated with $BaCl_2$. The $BaSO_4$ formed is then weighed, and the milligrams of SO_3 per 100 sq. cm. per day calculated.

The results so obtained may be taken as representing the activity of the SO_2 in the atmosphere in so far as it may cause decay of building-stone.

Results for SO_3 at Leinster Lawn.

Figure 4 gives the monthly figures for pollution by sulphur dioxide expressed as milligrams SO_3 per 100 sq. cm. per diem. The graph (upper line) shows a well-marked minimum in the summer months and maxima in January and December when coal consumption is at its highest. The continuous line graph gives the average monthly shade (suspended impurity) over the same period, and shows

a remarkable if somewhat fortuitous parallelism with the SO_3 graph. A few measurements of SO_3 were made in the garden of Church Villa, Rathmines, and the results are given in the short graph. The figure for SO_3 in Rathmines for October is 0·33 of that at Leinster Lawn, and for November and December about 0·4 of the figures at Leinster Lawn.

A comparison of the pollution due to sulphur gases in Dublin with that in other towns is shown in figure 5.

The curve for Dublin lies about midway between those for Glasgow and Cardiff, while that for Westminster Bridge shows an extraordinarily high degree of pollution by sulphur dioxide in the winter months.

From the results so far obtained it may be stated that the pollution of the atmosphere at Merrion Street, Dublin, is similar to that prevailing in some industrial centres in Great Britain. Morning and evening fogs appear to have become more frequent here than some twenty years ago. The residential area of the city has increased remarkably in the past decade, and it will be interesting to see how pollution will vary in the future. The subject is one of importance from the view-point of both economics and health, and one would gladly see a more extended study of the subject being carried on by observations at a number of centres in the city and suburbs of Dublin. In this connection it is good news to hear that observations for 1939 have been commenced by members of the chemical staff at the Agricultural College, University College, Dublin, at Glasnevin.

The authors wish to express their thanks to the Royal Dublin Society and University College, Dublin, for grants which made the work possible, to the Ministry of Defence for permission to set up apparatus in Leinster Lawn, to Mr. A. Hassard, M.A.I., for permission to set up apparatus in his garden at Rathmines, and to Dr. J. S. Owens, of the Atmospheric Pollution Research Committee, for his courtesy and helpful advice in the early stages of the experimental work.

No. 9.

THE OVULE, GAMETOPHYTES, AND PROEMBRYO IN *SAXEGOTHAEA*.

By W. J. LOOBY and J. DOYLE
(Botany Department, University College, Dublin).

(Plates 2 and 3.)

[Read November 22, 1938. Published separately May 5, 1939.]

The existing coniferous genera may best be considered as survivors of many lines of descent which took their origin, perhaps, in Palaeozoic time. In any large phylum of such an age it would always be difficult to trace these lines with any degree of probability, but this difficulty is all the greater in the conifers owing to certain serious gaps in our knowledge. It is perhaps doubtful if the relationships of the living forms will ever be properly clarified, but, as long as these gaps exist, many current ideas can be little more than guesswork. Our knowledge of the fossil history of the conifers, for example, is still very inadequate, and further information is particularly needed on the structure of Palaeozoic coniferous cones to permit a proper understanding, based on fact, of that central problem—the morphology of the conifer strobilus. It is to be hoped, therefore, that much new light will be thrown on this problem by the work upon which Dr. Florin of Stockholm is at present engaged. Further, there are still many genera about whose life-history we are at present entirely ignorant, or of which we possess only a fragmentary knowledge. These genera are:—*Acmopyle, Amentotaxus, Callitropsis, Diselma, Fokienia, Glyptostrobus, Microbiota, Saxegothaea* and *Taiwania*. In many other genera, also, important details have yet to be worked out. The genera mentioned, however, being mostly monotypic and endemic, may possibly be relict or more primitive forms, and, if so, must be regarded as important in the groups to which they severally belong. Most of these are unfortunately not available for study, at least in W. Europe, as they are not yet in cultivation or are represented only by very young specimens. *Saxegothaea*, however, is represented sparingly in gardens by plants of maturer age, and is thus, for the present, the last and only coniferous genus available in W. Europe of whose life-history we are all but completely ignorant. As is well known the genus is represented by the single species *S. conspicua* Lindl.

It is now thirty years since three papers were published on *Saxegothaea* by Norén (1908), Stiles (1908), and Tison (1909). The structure of the pollen cone is described in all, but Norén also gives a full account of the pollen grain up to the time of shedding. Nothing further need be added for the present to

this account by Norén, which has been examined and is substantially correct. The structure of the ovule, with its peculiar projecting nucellus, was described at the same time, Norén and Tison also giving some account of its development and of the phenomena of pollination, our knowledge of which has more recently been extended and corrected by Doyle and O'Leary (1935). But the only definite record bearing on the rest of the life-history is the mention, by both Norén and Tison, of a single large spore mother-cell in the ovule surrounded by a marked tapetum. Norén probably saw the young prothallus at the parietal nucleate stage, makes a completely incorrect reference to the gynospore membrane, and refers to a photograph (his Fig. 24) as showing archegonia resembling those of *Podocarpus,* a photograph, however, in which nothing definite can be seen.

The results here described were obtained from a specimen growing at Kilmacurragh, Co. Wicklow. Two very good trees of *Saxegothaea* are growing in this estate. One of them, however, coned much more sporadically than the other, and was only useful for occasional comparison. *Saxegothaea* proved a rather difficult subject. The number of cones formed in most years were very few, and of those pollinated in any one year fewer still survived to complete their development in their second season. Except in the occasional good years each cone seldom matured more than two seeds, commonly only one, although in 1935, a year following a profuse coning, as many as seven fertile developing ovules were found in a single cone. The material has been under observation since 1930, when the first collections were made. Some of these were worked through by Mr. Brendan Lawler (then a student in the Department, now Fr. B. Lawler, S.J.). The results obtained, while very incomplete, seemed at the time sufficient to indicate at least the approximate dates of some of the critical stages. Occasional use has been made of some of his preparations, and we wish here to make grateful acknowledgment to him. The most successful collections were those of 1935–6, since when only a few cones have appeared, probably related to the seasonal fact of a succession of cool wet summers. Owing to the constant necessity of sparing the material certain stages are not as complete as could be desired, particularly the early development of the prothallus. These gaps, which are of lesser importance, can be filled at the next good coning year, but it seems advisable to put on record now the main essentials of the life-history, all of which are available.

The Gynostrobilus and Gametophytes (up to the winter resting stage).

Saxegothaea, as is well known, develops a true ovulate cone, each fertile scale carrying a single ovule. Dallimore and Jackson (1931) mention two ovules on a scale, but this is obviously a misprint. The cone appears first as a terminal bud on smaller branches. Norén suggests that branches are commonly staminate or ovulate, but on the Kilmacurragh specimens practically every branchlet carrying an ovulate bud carries young staminate cones also.

Some stages in the development of the ovule are drawn in outline in Fig. 1 A–G. Both Norén and Tison have also published drawings of stages in ovular development, but this series clarifies and corrects their drawings, and incidentally shows the relative size of the ovule and its parts at different stages in its development, as Fig. 1 B–G and Fig. 2 C are drawn to the same scale. When the bud first begins to swell at the latter part of April the ovule appears

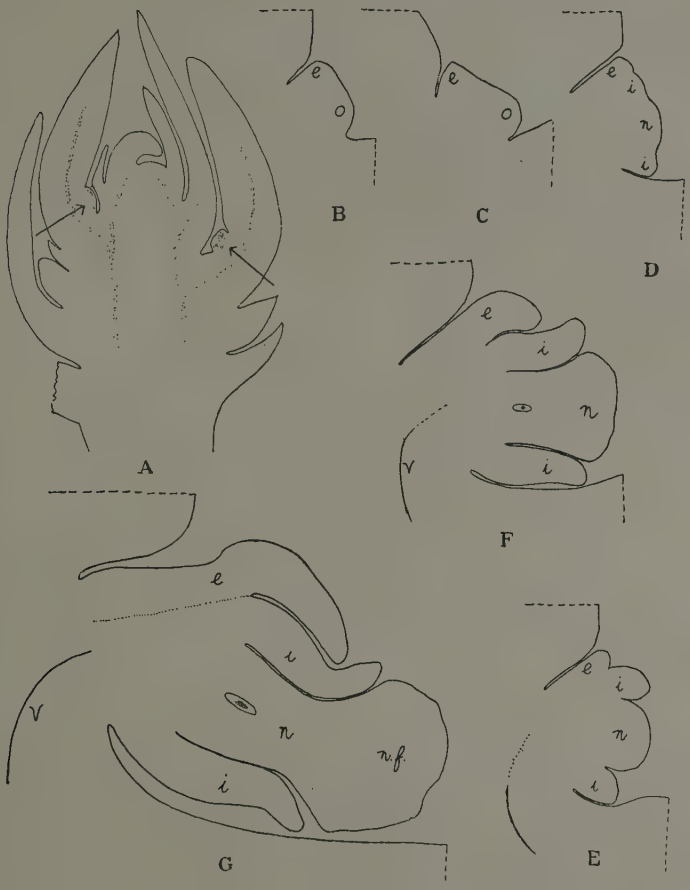

Fig. 1.—*Saxegothaea.* A. L.S. complete young cone to show position and relative size of earliest ovules examined. × 15. April 24. B, C. Outline of two such ovules to show epimatial and ovular regions at this stage. × 90. D. Outline of ovule to show origin of integument. × 90. May 23. E. Outline of ovule a little older than D. × 90. May 30. F. Outline of ovule at pollination. × 90. June 6. G. Outline of ovule as gynospore mother-cell matures and nucellus expands into the characteristic flare. × 90. June 15. In all figs. *e* is the epimatium, *o* the young ovule, *n* the nucellus, *n.f.* the nucellar flare and *i* the integument.

as a very small swelling some distance out on the scale. Two ovules appear cut in median section in Fig. 1 A, which is a longitudinal section of the complete cone. The swelling commonly is not symmetrical, but is slightly protruding or pointed at the upper side, as shown in Fig. 1 B. Fig. 1 C, although of the same date, is from another cone, and is slightly further advanced. The upper area represents the epimatium, the lower the ovule. Growth is slow, and the stage shown in Fig. 1 D is a month later, when the ovular part is differentiating out the integuments. The epimatium is thus developed at a very early stage, and the ovule could be described as being carried on the epimatium as well as on the scale. With no knowledge of the primitive podocarpean cone, or of the still earlier Palaeozoic one from which it was derived, it seems premature to interpret this development in terms, say, of the cone of *Pinus,* but it should be of interest to compare it with the early ovule and epimatium ontogeny in those podocarps in which the ovule seems carried fairly directly on the epimatium. It is hoped to follow this ontogeny in, at least, *Dacrydium,* the *Stachycarpus* section of *Podocarpus,* and in *Microcachrys.*

The ovule now develops rapidly. Fig. 1 E shows the form a week later, and Fig. 1 F that in the first week of June, when the scales open to receive the pollen. It has been pointed out elsewhere (Doyle and O'Leary) that the nucellar protrusion has not really a stigmatic function, but, as is the case with *Araucaria,* is related to the reception of the pollen tube. This appears all the more clearly from this examination of ovular development. It must be remembered that pollen is being shed continuously for some time, that the scales of the cone open while the cone is still small, and remain open and receptive for about ten days, during which the ovule and the flare grow considerably. But when the scales first open the nucellar flare is clearly small. Actually of many young cones examined about this period, all plentifully sprinkled between the scales with pollen grains, the ovule, figured in Fig. 1 F and Pl. 2; 1, was the most advanced. It was drawn and photographed because it also showed the earliest stage of the gynospore mother-cell. Most ovules are somewhat smaller, with the nucellus practically flush with the rim of the integument. The pollen may fall on the nucellus, as it may fall on the side of the ovule or elsewhere, but there can be no question of a special stigmatic function. Actually on the scale around the neighbourhood of the ovule there can be seen a zone of slightly translucent tissue. It would appear that a pollen grain falling anywhere on this area, or on the ovule itself, can germinate, and its tube successfully reach the nucellus. The tubes may thus be of greatly varying length according to the distance, which may be very short, of the pollen from the growing nucellus. During the succeeding week—up to the middle of June—the ovule increases further in size, and the nucellar expansion becomes definite, this period covering the maturing of the spore mother-cell, spore formation, and early spore germination. Fig. 1 G and Pl. 2; 2 show this relative increase.

The gynospore mother-cell appears as a single cell deep in a fairly uniform nucellus. It rapidly increases in size, and as it matures a definite "tapetum" or "spongy tissue" develops around it. The complete ovule is shown in Pl. 2; 2, and the mature mother-cell and tapetum from another ovule in Pl. 2; 3 at a higher magnification. This stage has already been figured by Norén, and is also shown by Tison (see his Figs. 3 and 8). It is clear that the well-known photograph from Norén's paper showing the scale of *Saxegothaea* with the ovule and its protruding nucellus is an oldish stage, and *after* pollination. Tison, however, shows a photograph of the ovule *at* pollination which is very similar to our Pl. 2; 1, and which also shows the young spore mother-cell. Tison, too, correctly states that the nucellus principally protrudes *after* pollination.

This distinct single gynospore mother-cell with specialized surrounding tissue seems to be characteristic of the podocarps. It has been also described in *Microcachrys* (Lawson, 1923 a), and in *Dacrydium* and four sections of *Podocarpus* (Sinnott, 1913). It has not been seen, but can be safely presumed from the stages available in *Pherosphera* (Lawson, 1923 b) and *Phyllocladus* (Young, 1910). The development of the tapetal zone, however, seems to be very variable in *Podocarpus*. Sinnott (1913) has recorded it in several *Eupodocarpus* species, although it is said to be absent in the species examined by Coker (1902)—*P. coriaceus*. On the other hand, although Sinnott reports that it is absent in the *Stachycarpus* species examined by him—*P. spicatus* and *P. ferrugineus*—yet recent personal examination shows that it is quite definitely present in *P. andinus*. Its definiteness depends to some extent on the maturity of the ovule and on suitable staining; it may have been overlooked in some cases. In general, however, it seems a normal podocarpean feature.

The spore mother-cell divides, and the lower cell divides again, giving in all a linear chain of three. A young stage is drawn in Fig. 2 A, and shows the dense tapetal cells passing abruptly into the vacuolate cells of the nucellus. The upper cell, which has not divided, shows early signs of nuclear degeneration. The middle spore may be obviously less important than the lower one, which, as in the case drawn, will clearly give the single prothallus; but these two may be of almost equal size and similar in appearance, in which case two prothalli may develop later, a phenomenon found quite frequently. The condition drawn is, however, definitely the normal. Once only was a full tetrad seen in which the upper nucleus had also divided, but, as a result of early degeneration in this upper cell, it is not quite certain if any wall or membrane was formed between the nuclei. A full tetrad has been described for *Microcachrys* (Lawson, 1923 a), *Dacrydium* (Sinnott), and for the *Stachycarpus*, *Dacrycarpus*, and *Eupodocarpus* sections of *Podocarpus* (Sinnott), although in *Eupodocarpus* there may sometimes be only three in the group. The spores have not been observed definitely in *Phaerosphaera* or *Phyllocladus*, but a linear group of three is possible (Lawson, 1923 b, Young). The spore number is thus a variable feature in the podocarps, as it is in other conifers; on the

other hand, the development of more than one spore, in many cases even to the formation of two mature gametophytes with archegonia as in *Saxegothaea,* has been recorded for all the genera except *Microcachrys,* and for many podocarpean species especially in *Eupodocarpus* (Sinnott, Coker, Stiles, 1912, Holloway, 1937).

The germination of the spore, at least when only one is present, shows a remarkable feature. The nucleus does not divide for some time. Fig. 2 B shows the young prothallus with still only one nucleus in mid-July, but with

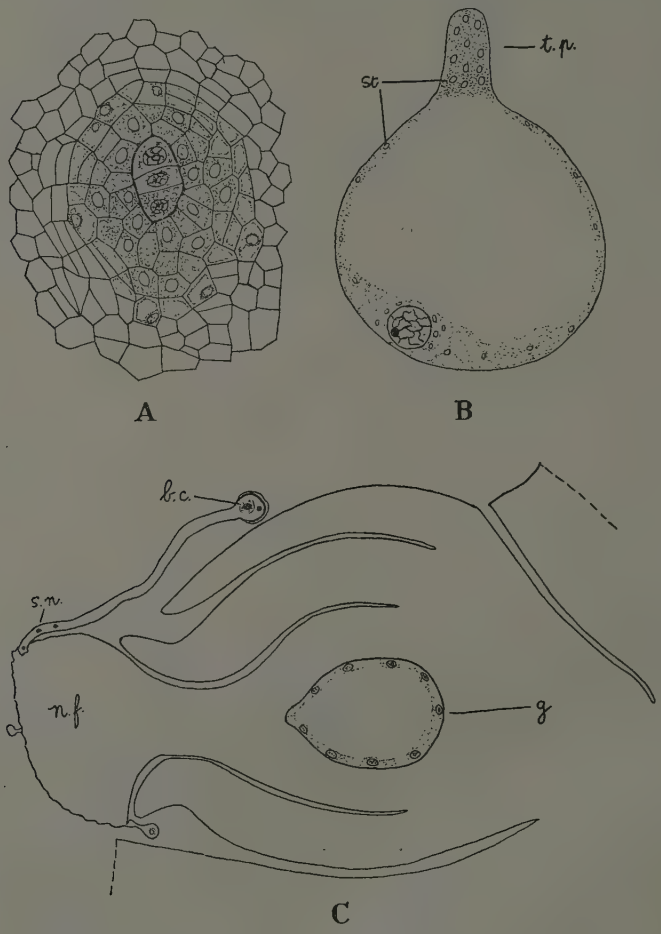

Fig. 2.—*Saxegothaea.* A. Young "tetrad" of three cells surrounded by tapetum. × 340. June 17. B, Young prothallus still uninucleate showing tent-pole protrusion, *t.p.* and starch grains, *st.* × 570. July 15. C. Outline of ovule in the resting winter stage,—*g,* prothallus; *n.f.* nucellar flare; *b.c.,* body cell in grain with another nucleus; *s.n.* sterile nuclei which have already passed to tip of pollen tube. × 90. Sept. to April.

a sharp protrusion which develops in the upper part, and contains dense protoplasm and many starch grains, which are also scattered at this stage throughout the thin layer of protoplasm which surrounds the large central vacuole. This peculiar protrusion, while remaining small, develops with the prothallus practically up to fertilization as a small "tent-pole." Pl. 2; 12 shows it when the prothallus in June of the next season has formed archegonia about ten days before fertilization. In *Acmopyle* Sahni (1920) has described a structure perhaps homologous with this, but much more massive.

The young prothallus grows slowly, and by the late autumn reaches the parietal nucleate stage, as shown in Fig. 2 C, with probably 128 nuclei. In this condition it passes the winter, resuming growth in the succeeding May. The pollen grain in the meantime germinates, shortly after falling on the scale, and, by mid-July at latest, the pollen tubes reach the nucellus. The tip, as already described elsewhere (Doyle and O'Leary), becomes embedded a few cells deep in the nucellus, and remains at this stage through autumn and winter till late spring in the following year. The free nuclei in the grain pass along the tube, but the body cell always, and apparently frequently another nucleus which is probably the stalk nucleus, remain in the grain during the whole resting period. This later movement of the body cell has been described for *Phyllocladus* (Young), *Podocarpus* spp. (Sinnott), and *Dacrydium* (Stiles, 1912). In *Dacrydium cupressinium,* where development is spread over two growing seasons, the body cell lies in the tube during the whole resting period. This probably also happens in those species of *Podocarpus* which have a long resting stage, but is not specifically mentioned by Sinnott. Recent personal observation shows that the body cell does so remain in the pollen-grain during the resting season in *P. andinus.* On the other hand, although the pollen grain of *Microcachrys* is definitely podocarpean, yet the body cell does not show this lingering in the grain if Lawson's (1923 a) account is correct. As the grain of *Phacrosphera* (Lawson, 1923 b) is definitely exceptional in the absence of prothallial cells and, consequently, free prothallial nuclei, no comparison can be made with it; indeed its structure and general life-history differ so much from the other podocarps that it is doubtful whether its relations really lie with this group. But in *Microcachrys,* with its podocarpean grain and its cone-scales and ovular arrangement so like *Saxegothaea,* it is undoubtedly surprising to find this point of difference.

Fig. 2 C thus shows the general winter condition of the ovule and the two gametophytes. From a comparison with Fig. 1 G it is clear that the whole ovule has grown, but that there is no relative increase in size in the nucellar flare. The cells of its outer layer or two, however, become larger, more irregular, and definitely vacuolate, contrasting strongly with the densely protoplasmic and compact cells of the rest of the flare. Incidentally, the fine spiral thickenings described by Norén in the cells of its neck are not limited to this area, as he says, but are found freely elsewhere. Further, as already stated, only one or two fertile ovules were found in any cone in the succeeding year.

This sterility of most of the ovules is already apparent during autumn and winter. In spite of successful pollination, dissection of the cones during these periods shows a brown withered speck on most of the scales—the shrivelled nucellar flare of degenerating ovules. Those whose flare was not reached by tubes always die, but the shrivelled remnants of tubes can be seen in many others. Those few ovules in each cone which have successfully developed to the stage of Fig. 2 C show at once, as the nucellar flare can be easily seen as a swollen semi-translucent mass, frequently with masses of tubes attached to it like a minute Medusa.

Tison describes, and illustrates in his Fig. 16, a layer of tissue on the upper surface of the scale which passes below the ovule to continue and end with the epimatium, as if the epimatium were a distinct organ carrying the ovule and which had fused with the scale. This does not seem to be a fact. The tissue on the upper part of the scale and above the vascular strand is a little different in structure from the tissue below it, at least in the central area; but this tissue leads, following the bundle, to the base of the ovule. Pl. 2; 2, 4 (the latter a section of an older ovule with the functioning spore just germinating), as well as the younger stage, Pl. 2; 1, show clearly how the epimatial tissue stops fairly abruptly; but they also show, particularly Pl. 2; 4, the peculiar relation of the attachment of ovule and epimatium to one another and to the scale. Until the rapid onset of the relative growth of the ovule in the next season this relation can be clearly seen. The longer and strongly vacuolate cells of the epimatium tissue contrast markedly with the cells of the scale and with those at the base of the ovule.

There does not seem to be any other record in the literature of the resting stage of the female gametophyte in those podocarps with a long gap between pollination and fertilization. Again, from recent personal observation, it can be stated that, in *Podocarpus andinus*, at least, the resting stage is exactly that of *Saxegothaea*, viz.: a youngish vacuolate condition, approximately of the same size and with the same number of nuclei.

The Mature Gametophytes.

A. *The female gametophyte.*—No full account of the development of the female gametophyte from the vacuolate stage to the formation of archegonia is available for any podocarp. In *Pherosphaera* only an already cellular prothallus is described (Lawson, 1923 b), nor was the origin of the archegonia seen; in *Microcachrys* "alveoli" are described but archegonial formation was not observed; "alveoli" were seen in several species of *Eupodocarpus*, but reference to archegonial origin, both here and also in *Dacrycarpus, Stachycarpus,* and *Dacrydium,* is limited to the statement that each archegonium arises from a superficial cell. (Coker, Sinnott, Stiles, 1912.) Recently Holloway has given a description, accompanied by certain comments of which it is not easy to appreciate the implication, of the formation in *Phyllocladus* of primary

endosperm cells (or alveolar ingrowths), and mentions that the archegonia are formed early, two (or three) of the primary endosperm cells acting as archegonial initials.

In *Saxegothaea* these stages are also incomplete, partly because few collections were made during this period, partly because the young prothallus is very delicate. This stage is a very difficult one to fix in all conifers, but here the tendency to marked shrinkage in ordinary fixatives is extreme.

The vacuolate gametophyte resumes growth late in April or early in May, in which latter month alveoli or similar structures are formed. There may be, however, a difference of ten to fourteen days in different ovules. Details of alveolar development are not available, and from this point of view Fig. 3 A is merely diagrammatic; but it shows at the micropilar end a small cone-shaped zone of alveoli denser than the others. This zone plays a very definite part in the later prothallial development. The "tent-pole" is quite distinct. In addition, there surrounds the growing gametophyte a one-layered tapetum of fairly large cells which are already showing signs of degeneracy in the thinning out of their protoplasm. This layer has developed from the tapetal zone in which the functioning gynospore mother-cell was embedded. It makes immediate contact on the outer side with the innermost layer of the nucellus, a layer which at this time is formed of very narrow, badly disorganized cells. As further growth proceeds the tapetum gradually collapses. The duration of the tapetal zone in other podocarps in which a tapetum occurs is variable. In *Microcachrys* and *Pherosphaera* (Lawson, 1923 a, b) it disappears shortly after the first nuclear divisions in the germinating gynospore, while in some species of *Eupodocarpus* it persists apparently till the gametophyte is almost mature (Sinnott). In *Phyllocladus* and *Dacrydium* no definite statements are available as to the stage at which it disintegrates.

The further development may perhaps be followed from Fig. 3 B. Some of the upper group of more distinct alveoli (Fig. 3 A) function as archegonial initials, which are long and thin from the beginning. The others undergo rapid division. The alveoli in the rest of the gametophyte become cellular also, the growth being slow in these regions, the cells large and thin-walled; but the upper region functions like an extended meristem. The divisions, rapid and continuous and accompanied by cell expansion, produce a marked growth extension in the micropilar direction, the archegonia elongating in pace with it. This upper zone ultimately contributes about two-thirds of the mature prothallus and produces in the latter a very definite structure, as shown in outline in Fig. 3 C. The archegonia are very long and are surrounded by a zone of cells, rather narrow and rectangular, which continue down, as a more distinct cone of tissue, well below the archegonia. These cells, especially in the sub-archegonial cone, early become multinucleate. The rest of the prothallus is still composed of fairly large, uninucleate, vacuolated, thin-walled cells, whose formation from the original alveoli can be followed in most of the tissue by their regular radial arrangement. The whole often gives the impression of cells radiating in all directions from a point near the base of the cone but at

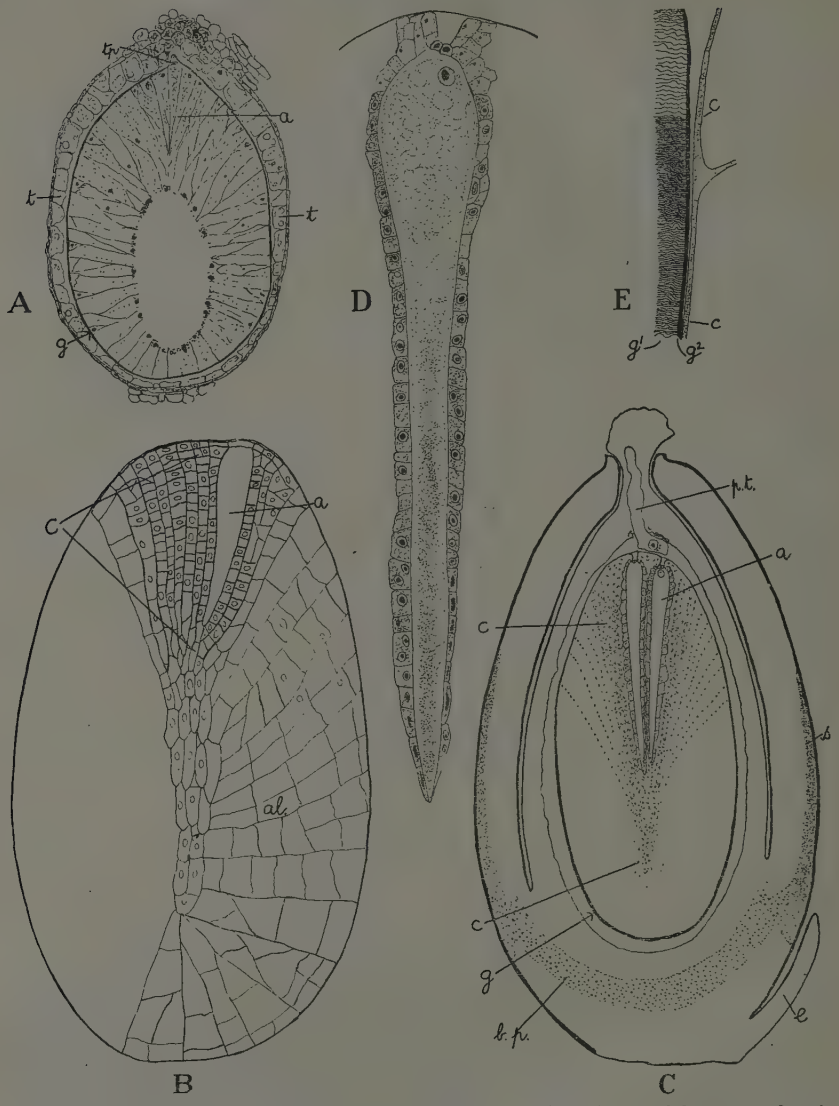

FIG. 3.—*Saxegothaea.* A. Young alveolar prothallus showing the special group of apical alveoli, *a*; the tent-pole, *tp*; the tapetum *t*; and the gynospore membrane, *g*, already well developed. × 55. May 21. B. Slightly older prothallus showing the apical cone of meristematic tissue, *c*; one archegonium, *a*, developing from an alveolus; the thin-walled radially arranged tissue, *al*; and the central zone of cells which help to form the sub-archegonial cone. Very diagrammatic. × 90. May 21. C. Outline of general ovular structure when the pollen tube, *p.t.*, with contained body cell has just reached an archegonium, *a*, embedded in the cone of tissue, *c–c*; *e*, epimatium; *g*, gynospore membrane; *b.p.*, sclerotic basal plate; *s*, stony layer of integument. × 25. June 8. D. Young archegonium. × 90. June 12. E. Gynospore membrane showing the two layers g^1 and g^2 and the wall *c* of the outermost cells of the prothallus. × 1000.

gradually increasing angles. The sub-archegonial cone is partly formed from cells cut off, in a manner unknown, from the ordinary alveoli, as shown by the central cells in Fig. 3 B. Many details are obviously lacking, but the general plan is of interest, as it dominates the whole structure even up to the later formation of the storage endosperm in the seed. The presence of a cone of denser multinucleate cells below the archegonia is mentioned by Sinnott in many species of *Podocarpus* (particularly well seen in *P. ferrugineus*) and in some species of *Dacrydium*. As the development, however, has not been followed out, it is impossible to say how far this phase in *Saxegothaea* may be peculiar to the genus. In at least those forms with long and large archegonia, namely, species of *Dacrydium* and of the *Stachycarpus* section of *Podocarpus*, a somewhat similar development might be expected, and an examination of this point is desirable. In general the female gametophyte structure is fairly typical of large groups. The mature prothalli and archegonia in the abietinean, cupressinean, callitrinean, and araucarian conifers are quite distinct. The corresponding structure in the more primitive podocarps may also prove equally distinct, not only in their mature form but in their development.

The size of the young archegonia relative to the whole gametophyte and ovule also appears from Fig. 3 C. When still very young they contain many large vacuoles, but shortly before the division to form the ventral canal nucleus the narrow part of the archegonium becomes filled with denser protoplasm, the under part towards the neck being still occupied by many vacuoles of varying sizes (Fig. 3 D and Pl. 2; 5). This whole upper area may be much more delicate, and with larger vacuoles, than is here shown. The archegonia are three (rarely two) in number (Pl. 2; 6) and are spaced fairly evenly, their necks opening into shallow pits arranged around the tent-pole projection (Pl. 2; 12). They lie slightly obliquely in a longitudinal direction, so that, in most cases, archegonia complete in their length for photographic purposes can only be got from somewhat oblique sections. The jacket cells are very distinct. They are formed from the neighbouring lines of rapidly growing cells in the young meristematic apical cone already referred to. At the stage before the entry of the pollen-tube, or if a pollen-tube does not reach the archegonium, the neck is only slightly sunk below the level of the gametophytic tissue, the small pit being covered with the gynospore membrane (Fig. 3 D, and Pl. 2; 5, 10, 12). In Pl. 2; 12 the whole gametophyte is younger, so that the necks are still practically flush with the surface. The neck is formed of one tier of cells 6–10 in number, 6–8 being common. The neck figured in Pl. 2; 9 not only shows the eight cells clearly, but also their manner of formation. Except in some of the more advanced species of *Podocarpus*, in which more than one tier is formed, this single-tiered neck of from four to ten cells has been described for all other podocarps. It should further be noted that the dense jacket cells do not continue at this stage right up to the neck, but stop about the shoulder. The ventral canal division soon follows (Pl. 2; 7), the ventral canal nucleus being usually fairly permanent. In many cases it may remain a conspicuous object in the upper part of the archegonium, as shown in Pl. 2; 11, although usually

it is smaller. It may, however, leave this position and pass well along the archegonium into the narrow part. The egg nucleus comes to lie somewhat down from the neck at about the widest part of the archegonium, and rapidly increases in size (Pl. 2; 11). The protoplasm also becomes much denser although far from uniform, being very granular and fibrillate at least in the fixed condition. The archegonium is now ready for fertilization (Pl. 2; 8).

This general type of gametophyte with a few large archegonia may well be primitive in the group. It is not found in *Pherosphaera* (Lawson, 1923 b), which, if a podocarp at all, is certainly a peculiarly unique one; and the archegonial complex of *Microcachrys* (Lawson, 1923 a) is surely a derived condition. On the other hand, it is found also in *Dacrydium* (Stiles, 1911) and in the more primitive *Stachycarpus* section of *Podocarpus* (Sinnott). In the admittedly more advanced *Eupodocarpus* section the larger number of small archegonia would appear to indicate another derivative line. It is not easy to decide from published reports what is the actual type of archegonium in *Phyllocladus*. The drawings of Holloway seem to show rather shorter archegonia in which, however, the egg nucleus behaves as in *Saxegothaea*, while an earlier line-drawing by Young (her Fig. 36) distinctly shows archegonia extending to the centre of the prothallus. If the archegonia in *Phyllocladus* are not exactly of the *Saxegothaea* type they must on the whole rather closely resemble it.

The gynospore membrane is thick, and develops at a very early stage. It is of the usual two-layered type, the exosporium being formed of radial fibrillae without, however, any capitate ends (Fig. 3 E). These fibrillae are delicate, but can be seen clearly with higher magnifications. In fixed preparations they appear sinuous, but this may be due to pressure resulting from the contractions in the ovule. If so, the membrane in nature may be even thicker than it is in prepared sections. About fertilization it is approximately 5μ in thickness at the base of the prothallus, thinning out a little above. Subsequently it seems to become finer, being a little over 3μ in mid-August. Fig. 3 E shows that the inner layer of the membrane is comparatively thin, apparently thinner, although much denser, than the walls of the immediately adjoining prothallial cells, at least in the older stages. The ovule from which this was drawn showed an advanced proembryo stage. In a much younger prothallus its cell walls would of course be correspondingly finer. The outer layer has been drawn with the fibrillae at one end of the membrane, much farther apart than they actually are; elsewhere they have been outlined roughly as they appear under the oil-immersion lens. In the thicker areas the fibrillae cannot be separately resolved if the stain is in the slightest too heavy. It is perhaps of interest that, although frequent reference will be found in the literature to the existence of a thick membrane in *Saxegothaea*, yet the only distinct statement made on the matter by those who have worked and published on the genus is that by Norén. It is not mentioned by Thomson (1905), and Norén actually states, though clearly in error, that the membrane is extremely fine (*von ausserordentlicher Feinheit*), and shows no "Querstreifung" under even the highest magnification.

A thick gynospore membrane occurs also in *Phyllocladus* (Young, Holloway), in *Dacrydium* (Thomson), and in *Microcachrys* (Lawson, 1923 a). In *Podocarpus* it is thick in the *Stachycarpus* section, and also in some species of *Eupodocarpus* but it is finer in *Dacrycarpus,* and apparently in many species of *Eupodocarpus* it has become so fine as to be difficult to demonstrate. (Coker, Stiles, 1912, Sinnott.) It is also thin in *Pherosphaera* (Lawson, 1923 b).

Fig. 3 C also shows the basal plate of sclerotic cells and the stony layer of the integument, a layer which develops acropetally. It is formed just a little in from the surface, leaving the outer cells as a very narrow belt, which gradually disorganizes, and appears in later stages as little more than a thin dark line. Both it and the sclerotic basal cells are thick-walled and pitted, and contain a large crystal. Complete ovules may be cut up to fertilization, but thickening becomes then more intensified, and the gametophytes must be dissected out later.

B. *The male gametophyte.*—The exact date at which the pollen tube resumes growth, and at which the body cell leaves the grain, was not determined. In the earliest stage available (May 22) the tube, containing a number of sterile nuclei, had already penetrated some distance into the flare, while the body cell, though still very small, had just reached the edge of the flare. The body cell thus rests in the grain for about eleven months. What factors or stimuli determine its ultimate movement? Growth is now more rapid. The body cell remains small, as the tube penetrates the neck, and is commonly drawn out to a narrow elliptical shape. It reaches the top of the gametophyte about the end of the first week in June, and approaches one of the archegonia, the whole tip and especially the body cell enlarging markedly. The body cell shortly before its division is shown in Pl. 2; 13. It is accompanied in this case by at least five sterile nuclei, of which three are shown. These are the tube nucleus, the stalk nucleus, and the derivatives of the prothallial cells, only one of which had apparently divided. If the first prothallial cell had also divided, the body cell may be accompanied by six sterile nuclei. The tube penetrates the gynospore membrane, enters the cavity above the archegonial necks, presses down actively against the top of the archegonium, and expands considerably. About this time the body cell divides, but it is not certain if it divides before entering the archegonial cavity or in it. The two archegonia in Pl. 2; 10 are of interest. No tube reached the one on the left, and the normal shallow pit, as earlier described, is quite obvious; the archegonium on the right shows how the tube presses down and expands. This is also shown, although not so clearly, in Pl. 2; 8 and in the drawing, Fig. 4 C. The peculiar position of the neck-cells at this stage, originally described and drawn by Young for *Phyllocladus,* and later by Stiles (1911) in *Dacrydium,* appears equally clearly in *Saxegothaea.* Sinnott's figure of *Podocarpus ferrugineus* (his Pl. 5; 2), although the point is not mentioned by him and the figure is a little diagrammatic, seems to show a similar condition. Recent personal observation shows it to be definitely present in the still simpler member of the *Stachycarpus* section, *P. andinus.* It can be seen in Pl. 3; 16 and in Fig. 4 C

that the neck cells are suspended by a membrane across the archegonium a little above its widest part. Pl. 2; 8 is a photograph of one of the sections from which Fig. 4 C was drawn. The neck-cells included in Fig. 4 C were a couple of sections further on in the ribbon, but this one was photographed to give the best general view of the whole archegonium and the male cells. Young originally suggested, and the suggestion is commonly agreed to, that the condition arises in this way. The pollen tubes reach the gynospore membrane before the prothallial tissue has completed its growth. This tissue then grows upwards, leaving the archegonia at the bottom of deep pits, which are occupied by the pollen tubes. The growth of this tissue, and the pressure of the pollen tube, result in a lateral stretching, first of the neck-cells and then of the adjoining jacket cells. The membrane represents the stretched wall of a surrounding ring of jacket cells. This explanation is quite feasible, but in *Saxegothaea* the mechanism may be different. It is shown in Pl. 2; 10—and this feature was met with so frequently that it is clearly a normal occurrence—that the female gametophyte tissue at this stage does not grow up actively for any distance above the archegonial necks. Actually some time after fertilization the archegonia may become submerged even in the absence of a pollen tube, but at this stage that has not yet taken place. It appears then that the pollen tube itself has been actively pressing down in cases such as are shown here and in Pl. 2; 8 and Pl. 3; 16. Reference to the younger archegonium (Fig. 3 D) recalls the fact that the true jacket cells extend just over the shoulder of the archegonium but do not reach to the level of the neck. As the archegonium gets older, even after the ventral canal nucleus has been formed, the protoplasm of the zone near the neck never becomes really dense, but remains more tenuous, partly vacuolate, partly thinly fibrillate, and even in the absence of a pollen tube may show signs of collapse and an in-shrinking of the neck. It seems then rather that, as the pollen tube presses down, this area of the archegonium yields before it, and that the actual archegonium wall becomes freed from the non-jacket cells below the neck and forced down, with the neck-cells, approximately to the level of the jacket cells proper. A small lateral expansion, associated with pollen-tube pressure, would easily yield room for the membrane to take up its position, as shown in Fig. 4 C. A more detailed re-examination of *Dacrydium* and *Phyllocladus* would be of interest. Close stages were not available in *Podocarpus andinus,* but on the whole it seemed to show the type described by Young. There is here, in any case, another point of gametophyte structure linking up *Dacrydium, Saxegothaea, Phyllocladus,* and the more primitive species of *Podocarpus.*

The division in the body cell produces the unique feature in *Saxegothaea*—two equal or sub-equal male cells. This feature seems of sufficient importance to merit a number of illustrations, from which the structure can be seen without elaborate description. The youngest stage, seen shortly after the division, is shown in Pl. 2; 14 and Fig. 4 A, a slightly older stage in Pl. 2; 15 and Fig. 4 B. As indicated in the figures, but with difficulty if at all to be seen in the

photographs, both of these show in the actual preparations a membrane crossing the middle plane. It may be little more than a delicate plasmatic membrane, or even only a faint cleavage plane. When young, each male nucleus has a large nucleolus, which becomes much smaller, probably even disappearing

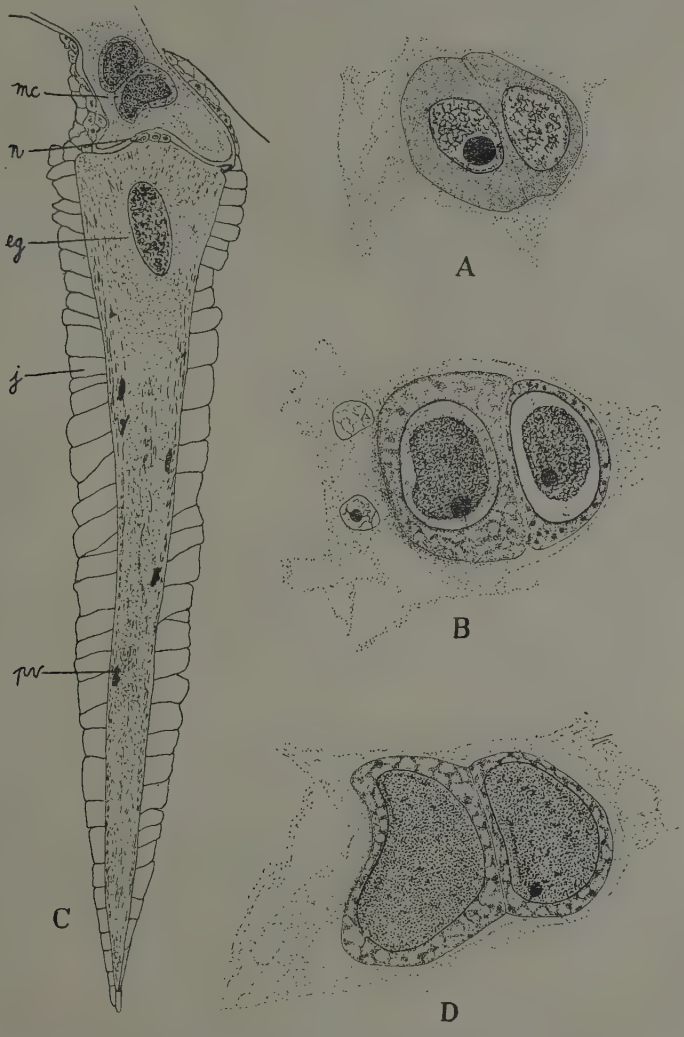

FIG. 4.—*Saxegothaea.* A, B. A young and a slightly older pair of male cells. × 340. June 8. C. An archegonium just ready for fertilization to show general form and arrangement—*mc,* male cells in pollen tube; *n,* neck cells suspended by a membrane; *eg,* egg nucleus; *j,* jacket cells; *pr,* protein bodies. × 90. June 13. D. The mature male cells of C enlarged. × 340. June 13.

entirely, at maturity, at which stage the nuclei are relatively very large, each surrounded by only a thin sheath of cytoplasm (Pl. 3; 16, 17, 19, 20). The two male cells never properly separate from one another, keeping in contact to a greater or less degree along the transverse plane. The double nature is clearly seen in Pl. 2; 10 and in Pl. 3; 21, which is a tangential section of the same pair shown in median section in Pl. 3; 17. In Pl. 3; 19 is shown an extreme case of inequality only met with once. This is not due to a lateral displacement of one male cell; the section is approximately median through both.

Two male cells such as these may safely be considered as primitive in the podocarps; but they have only elsewhere been described in *Microcachrys*. If Lawson (1923 a) is correct, the female gametophyte in this genus is very exceptional in a podocarp, but the existence of the two male cells in *Saxegothaea* suggests that the male gametophyte may, on the other hand, have retained a primitive feature. In all other podocarps, however, there has been recorded great, and even extreme, inequality in the male cells. The account by Young of *Phyllocladus,* and the accompanying drawings, shows two definite cells, one a small lenticular cell cut out by a membrane from the body cell, the main mass of which forms the other, the single large functioning one. An earlier account by Kildahl (1908), however, had described two equal nuclei lying in a body of protoplasm, somewhat similar to the condition in *Cephalotaxus* (Lawson, 1907). Holloway does not draw either the body cell or the male cells, but simply states that the body cell nucleus divides unequally into two *nuclei,* and subsequently gives drawings, of a rather diagrammatic nature, of various stages in fertilization in which certain nuclei are identified as the first and second male nuclei, the functioning and non-functioning ones, respectively. In his Fig. 28 the second male nucleus is very small, while the functioning one is as large as the egg nucleus; in his Fig. 31 the two male nuclei are much nearer in size, while the functioning one is only a fraction of the size of the egg nucleus; and in Fig. 27 he shows the fusion nucleus accompanied by the second male nucleus, which in this case is as large as the so-called functioning one in Fig. 28. Thus, without reference to the actual preparations from *Phyllocladus,* it is still uncertain what the structure of the male gametes really is; but, if only because of the clarity of the drawings, the condition described by Young may be temporarily accepted. In *Podocarpus* and *Dacrydium* (Coker, Stiles, 1911, Sinnott) one of the nuclei resulting from the division of the body cell nucleus is practically extruded, the other nucleus, with all (?) of the body cell cytoplasm, forming a single male cell with the second male nucleus partly embedded at its edge. This seems a very advanced reduction when one considers the extremely close similarity in the general gametophytic structures between *Saxegothaea, Dacrydium,* and at least the *Stachycarpus* section of *Podocarpus*; and certain body cell preparations in *Saxegothaea* have now raised doubts, admittedly very tentative, concerning this extreme reduction. Until an opportunity is available to examine fresh material, or the preparations from

which the published records were made, it may be advisable to give some explanation. The body cell of *Saxegothaea* in Pl. 3; 22 has a sterile nucleus partly embedded in it, and when this condition was first met with it was at once accepted as a podocarpean male cell arrangement. Sinnott's photograph (Pl. 9; 50) from *P. dacrydioides* is strikingly similar to this. He also gives a drawing (Pl. 5; 2) of *P. ferrugineus* in which this is repeated. These are the only illustrations given. His Pl. 9; 50 really looks more like a body cell, and, without some series of illustrations of body cells and male cells for comparison, might well be taken for a body cell. It is not clear from his paper how frequently this condition was met with, but it would seem to be considered the type for *Dacrydium cupressinum*, as well as for the *Stachycarpus*, *Dacrycarpus*, and *Eupodocarpus* sections of *Podocarpus*. Stiles (1912) gives two figures (Pl. 47; 29, 31) showing a somewhat similar condition in *P. macrophyllus*. Again there are not sufficient data to stamp the large cell here as a male cell; if it is it must be quite young, to judge from the size of the nucleolus and the extremely vacuolate condition of the archegonial apex in the Fig. 31. Stiles (1911) also figures a somewhat similar condition for *Dacrydium Colensoi* (his Fig. 4, p. 345). This figure is a comparatively simple line drawing, and thus is of little help in settling the doubt. Unfortunately in these two latter cases, at any rate, the original slides are no longer available for examination. Coker's figures for *Podocarpus coriaceus* (Figs. 12–16), on the other hand, look much more striking, as he draws and compares the body cell with the male cell arrangement. In this case male cells were only seen twice, and the functional one figured is very different in appearance from that given by Sinnott for *P. dacrydioides*. Personal observations in *Podocarpus andinus* were very limited owing to the fewness of the ovules available. The only stage seen was one which could be interpreted in terms either of Pl. 3; 22 or of the *Podocarpus* condition as described by others; the stage seen was definitely a young one. Thus, as far as *Podocarpus* and *Dacrydium* are concerned, there is only the single drawing of the body cell of *P. coriaceus*, made by Coker in 1902, to permit any comparison with stages in the organization of the gametes. It is quite probable that a rather specialized genus like *Podocarpus*, and particularly the more advanced members of it, have developed an extreme inequality of the male cells, but, if any doubt exists, it is necessary to settle the question with a suitable series of stages. It is hoped to do this at least for *Podocarpus andinus* this year.

In *Saxegothaea*, at any rate, the gametes just before fertilization are as shown in Pl. 2; 8 and Fig. 4 C, D.

Fertilization and Proembryo Formation.

Some phases of this stage are still uncertain, but they are mainly cytological and do not affect the main sequence.

It is probable, but not certain, that the contents of the pollen tube are discharged through the archegonial neck. At least one male cell and a number

of sterile nuclei enter. The second male cell may frequently be seen degenerating just outside the archegonium (Pl. 3; 26), but perhaps as often a second male nucleus can be seen inside, as appears in the top right corner in Pl. 3; 23. The fusion nucleus is large, about half as big again as the egg nucleus in Pl. 2; 8, and of a loose coarse reticulate nature. The fusion between the male and female nuclei seems to be complete, and at the first division after fertilization no indication could be seen of separate male and female spindles. The actual spindle is quite small in relation to the large fusion nucleus (Pl. 3; 23), and is intranuclear, the remnants of the original nucleus slowly disorganizing into the surrounding cytoplasm. The second division follows, and in approximately the same position. This stage is shown, though not very clearly, in Pl. 3; 18, but this was the only example obtained in which any part of the two divisions were visible in one section in the same plane. One of the four resulting nuclei appears in position in Pl. 3; 26. All through these stages the whole protoplasm mass would well repay proper cytological study. The cytoplasm in the rough fixed state shows great variations and changes in structure, densely and finely granular, fibrillate, strongly striate, and the like; while the organization of the small division from the huge fusion nucleus, leaving the main mass of it irregular in shape but with structure apparently little changed, should present a nice problem. It was difficult to follow the immediate movements of the four embryonic nuclei. In addition to these four nuclei there may be present, as a maximum, eight nuclei—four prothallial nuclei, a tube nucleus, a stalk nucleus, a ventral canal nucleus, and the second male nucleus. There are also commonly developed at this stage deeply staining cytoplasmic areas, "protein vacuoles," which are sometimes hard to distinguish from true nuclei; while other cytoplasmic aggregations of striae and granules may present a strikingly nucleus-like appearance. In general, however, it is clear that the four embryonic nuclei pass gradually to the pointed archegonial base. In Pl. 3; 28 and Fig. 5 A these are shown with another smaller nucleus following. Fig. 5 B shows one example of a multinucleate condition at this stage in which it is impossible to distinguish the embryonic nuclei. The clump of small nuclei towards the upper centre probably are some of the prothallial nuclei, while towards the apex the two larger nuclei may be derivatives of a ventral canal nucleus or one of them may be a second male nucleus reduced in size. With the material available it was impossible to disentangle the nature of all these nuclei, but, in the great majority of preparations in which the embryonic nuclei were approaching the pointed base of the archegonium, there was no difficulty in recognising them—as far as this stage is concerned Pl. 3; 28 is a typical case. The difficulties really lay between their first formation and their arrival at this later position. One point, however, is worth further mention. *Saxegothaea* resembles the species in the *Stachycarpus* section of *Podocarpus* (Sinnott) not only in the large size of the archegonia, but in the details of behaviour after fertilization,—the intra-nuclear first division, the second division following in the same position, and the passing of the four embryonic nuclei to the archegonium base. In *Eupodocarpus* on the other hand

the two nuclei resulting from the first division pass to the base where the second division occurs. Now, in the *Stachycarpus* spp., Sinnott describes the four embryonic nuclei passing to the base in a special sheath of dense protoplasm, although the subsequent fate of this sheath is not made clear.

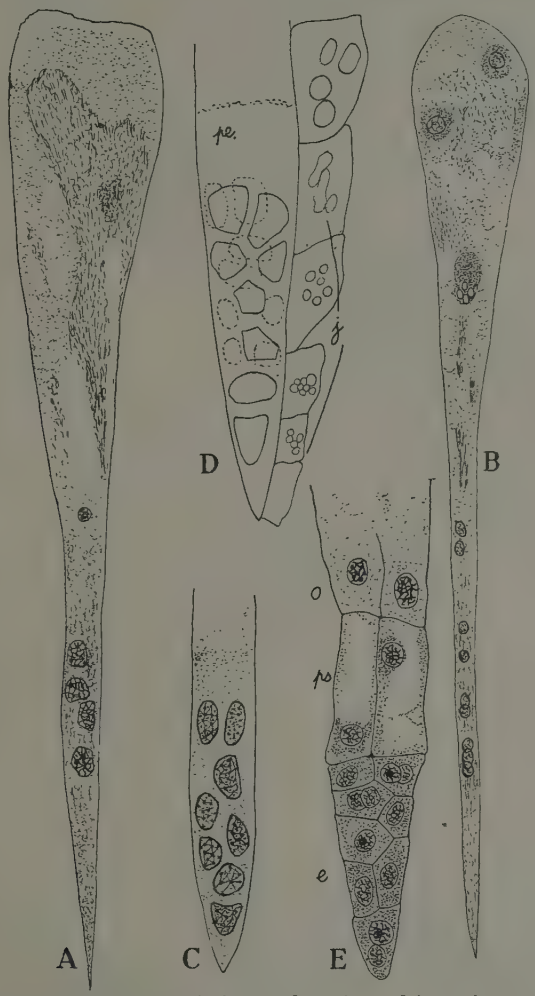

Fig. 5.—*Saxegothaea.* A. The typical four embryonic nuclei passing to the base of the archegonium followed by a smaller sterile one. × 90. June 21. B. Archegonium with a number of nuclei passing to the base among which it is not possible to distinguish the embryonic nuclei. × 90. June 20. C. An 8-nucleate proembryo. × 320. June 20. D. A 16-nucleate proembryo, *pe*, showing the general lie of the nuclei, with on the right jacket cells, *j*, showing the result of nuclear fragmentation. × 320. June 21. E. Walled proembryo showing the open tier, *o*, the prosuspensors, *ps*, beginning to elongate and the embryonic group of cells, *e*. × 320. June 21.

A somewhat similar appearance was frequently seen in *Saxegothaea*; but it was not possible to decide if the nuclei associated with it were the embryonic ones, nor was this cytoplasmic zone ever seen to get any distance down the narrow part of the archegonium. It sometimes contained four nuclei, sometimes two with other nuclei close to it. The preparation figured in Pl. 3; 24 had two nuclei within and two just outside. Now about this period, sheaths tend to form round any of the nuclei in the sense that the cytoplasm in their immediate neighbourhood becomes denser and more finely granular than the coarse and striate protoplasm elsewhere, as shown by the upper nuclei and the median clump in Fig. 5 B. The general impression was, however, obtained that after the second division the four embryonic nuclei may bring with them for some distance in their movement to the base a certain amount of the mass of cytoplasm in which they are enclosed, but sooner or later pass out from it and continue their passage as free nuclei. Although apparently of less definiteness and importance in *Saxegothaea,* its presence is a further point of similarity with the *Stachycarpus* spp.

A third simultaneous division follows (Pl. 3; 27), giving rise to eight free nuclei. The four divisions are strung out much in the same position as the nuclei in Pl. 3; 28, and the arrangement at the eight-nucleate stage corresponds with this, as shown in Fig. 5 C. A comparatively small zone of cytoplasm is now controlled by the nuclei, the main mass of cytoplasm in the rest of the archegonium rapidly thinning out and showing signs of disorganization. How small a portion of the whole archegonium forms the proembryo at this stage is shown by the deeply staining area in Pl. 3; 29. These nuclei divide again—this division was not seen, so it cannot be said if it is a simultaneous one—and a sixteen nucleate stage is formed. This is shown in median section in Pl. 3; 30, and diagrammatically, to show the lie of whole group of nuclei, in Fig. 5 D. Clearly, walls are not formed in connection with the spindles of this final division, as the nuclei are back to the resting stage and no traces even of spindle remnants can be seen. Walls do form, however, shortly after, but, as the actual stage was not obtained, nothing can be said on the mechanism of their formation. A certain degree of tiering takes place, resulting in (*a*) an open tier of nuclei, three to five in number, (*b*) a tier of cells again three to five in number which will form the prosuspensors, and (*c*) a conical group of embryonic cells six to ten in number. The young proembryo at the stage when the prosuspensors are beginning to elongate is shown in Pl. 3; 31 and Fig. 5 E. An apical cell is prominent, and it will be seen that it and some of the other cells are shown as binucleate. These cells were uninucleate when wall formation took place, and have since become binucleate by later division. At this time starch grains develop in the cone of tissue described under the development of the gametophytes; grains are found in cells well up along the sides of the archegonia, but particularly in the neighbourhood of the proembryo and the more solid part of the cone below the archegonia. The starch grains appear in Pl. 3; 30 and 31. These fertilization and proembryo stages take place in the third week of June.

In a subsequent paper it is hoped to deal in full with the embryogeny of *Saxegothaea* and its relation to that of other podocarps, so no further consideration need here be given to any comparison of proembryo development in them.

Saxegothaea shows another point of interest which may occur in other conifers, but which does not seem to be recorded. The jacket cells, like all the cells of the central cone, early become bi- or trinucleate. At the time of the first post-fertilization division the nuclei in the jacket cells of approximately the upper third of the archegonium undergo simultaneous division also. The first division is rather easily overlooked, and attention was called to it on a couple of occasions by the appearance of this simultaneous division in the jacket cells. As these may contain more than one nucleus there may be a corresponding number of spindles in each cell. A tangential section of a group of jacket cells at this stage is shown in Pl. 3; 25. At the third division a corresponding appearance is seen in the jacket cells of the lower part of the archegonium. A couple of cells of the jacket with nuclei in division may perhaps be seen on the right of the archegonium in Pl. 3; 27. The second division was seldom seen, but it is possible that it is also accompanied by simultaneous divisions in the jacket cells about the middle region of the archegonium. Although quite definite over a large extent of cells during at least the first and third divisions, occasional groups of jacket cells may be seen with the nuclei dividing during the whole post-fertilization period up to the full proembryo formation. As a result these cells become multinucleate, and, when a little older, show further fragmentation of the nuclei. The drawing, Fig. 5 D, shows in outline some of these nuclear clumps in the jacket cells on the right.

In the course of this paper comparison has been made between various stages in *Saxegothaea* and those in other podocarps. It is hoped in the succeeding paper on the embryogeny in the genus to consider what conclusions may tentatively be drawn from these comparisons about the Family as a whole in the light of the fuller knowledge gained from *Saxegothaea*. As no information is available concerning the developmental history of *Acmopyle* this genus has not been included in these comparisons.

Summary.

1. In ovular development, the epimatium is prominent at a very early stage. The nucellar development in relation to pollination is further clarified.

2. The female gametophyte develops a small tent-pole. The main mass of the prothallus is formed from an upper conical zone of cells derived from early alveoli.

3. The archegonia, three in number, are large, long, and pointed, and originate from apical alveoli.

4. Two sub-equal male cells are formed which do not completely separate.

5. Two post-fertilization divisions take place in the upper part of the archegonia, the four nuclei migrating to the base.

6. Two further divisions give a proembryo of 16 nuclei which are roughly tiered to form an embryonic clump of cells, a zone of prosuspensors, and a tier of free nuclei.

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EXPLANATION OF PLATES.

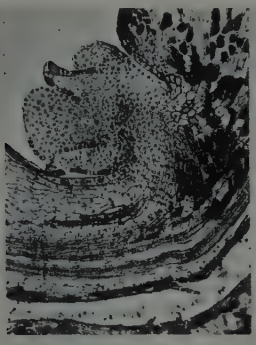
Plate 2.

1. Section of ovule on scale at pollination, showing also young gynospore mother-cell. × 62. June 6.
2. Section of ovule, slightly older, showing growth of nucellus, mature spore mother-cell, and tapetum. × 62. June 15.
3. Section of nucellus showing mature spore mother-cell and tapetum. × 265. June 15.
4. Section of ovule to show relation of ovule, epimatium, and scale; functional spore germinating. × 62. June 15.
5. Longitudinal section of young archegonium. × 62. June 12.
6. Transverse section of part of prothallus showing three archegonia with jackets. × 124. June 12.
7. Longitudinal section of archegonium showing division forming the ventral canal nucleus. × 265. June 9.
8. Longitudinal section of archegonium very shortly before entry of the pollen tube contents, showing mature male cells and mature archegonium. × 62. June 12.
9. Transverse section of neck of archegonium. × 265. June 12.

10. Section of upper part of prothallus just before fertilization showing on the left an archegonium with no pollen tube and on the right the relation between an archegonium and its pollen tube, the latter with two male cells. × 124. June 12.
11. Longitudinal section of archegonium showing large permanent ventral canal nucleus and position and size of egg nucleus when ready for fertilization. × 124. June 19.
12. Section of upper part of young prothallus showing "tent-pole" and young archegonium. × 132. June 8.
13. Body cell in section with three sterile nuclei. × 265. June 10.
14. Young male cells in section. × 265. June 8.
15. Slightly older male cells. × 265. June 8.

PLATE 3.

16. Longitudinal section of upper region of another archegonium with pollen tube and male cells. × 124. June 12.
17. The same at a higher magnification. × 265. June 12.
18. Second sporophytic division; lines indicate position of actual mitoses. × 132. June 20.
19. Male cells markedly unequal. × 124. June 12.
20. The male cells from 8 at a higher magnification. × 124. June 12.
21. Tangential section of the male cells in 17 showing separating groove. × 265. June 12.
22. Body cell with embedded sterile nucleus. × 265. June 10.
23. First division of fusion nucleus; second male nucleus inside archegonium. × 265. June 19.
24. Embryonic nuclei leaving upper part of archegonium, associated with a special mass of cytoplasm. × 124. June 19.
25. Simultaneous divisions in jacket cells accompanying first sporophytic mitosis. × 265. June 20.
26. Second sporophytic division just completed, only one small group of compacted chromosomes out of four showing, and indicated by the arrow; male cell remnants outside. × 124. June 19.
27. Third sporophytic division at base of archegonium; simultaneous divisions in jacket cells to the right. × 265. June 20.
28. The four embryonic nuclei near the base of the archegonium followed by a sterile one. × 124. June 21.
29. Longitudinal section of archegonium to show how small a zone of its base goes to form the proembryo; the small dark mass is an eight-nucleate proembryo heavily stained. × 62. June 19.
30. Proembryo at the 16-nucleate stage, walls not yet formed between them. × 265. June 20.
31. Young walled proembryo in section showing open tier of nuclei above, prosuspensors beginning to elongate, and group of embryonic cells, some of which are binucleate. × 265. June 21.



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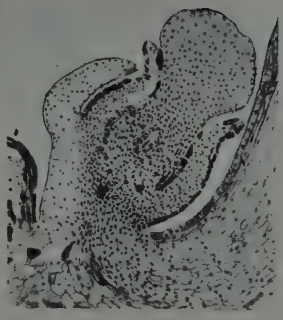

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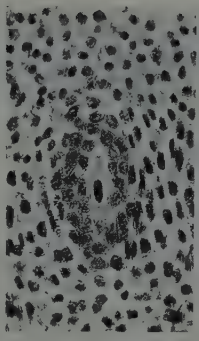

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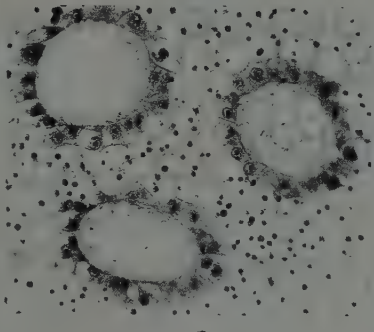

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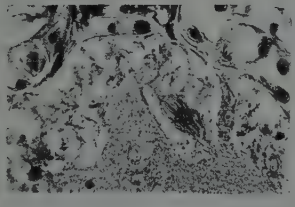

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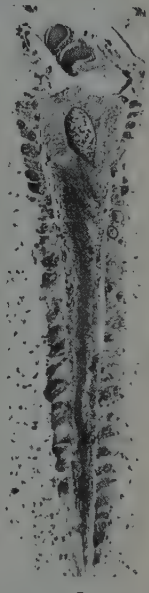

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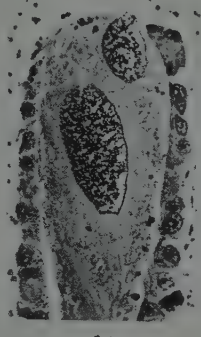

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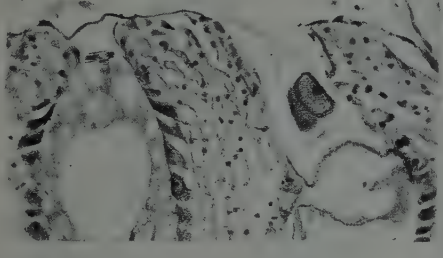

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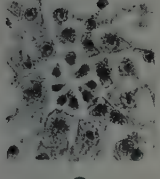

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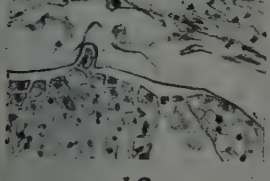

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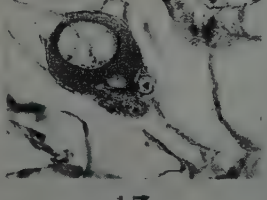

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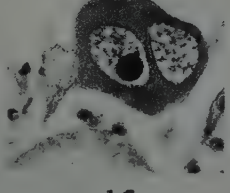

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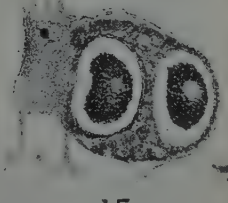

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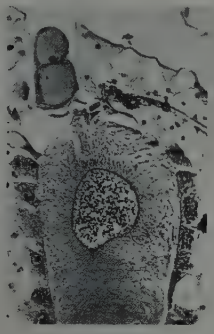

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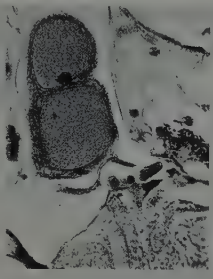

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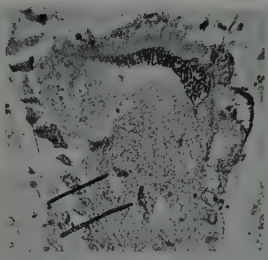

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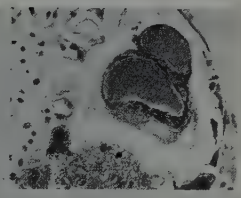

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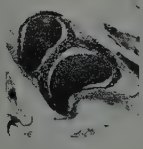

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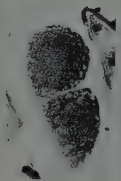

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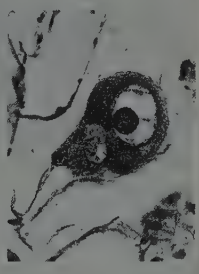

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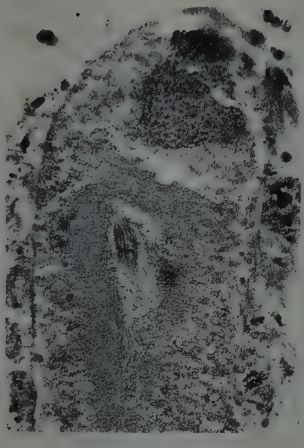

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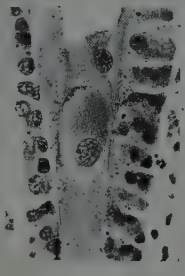

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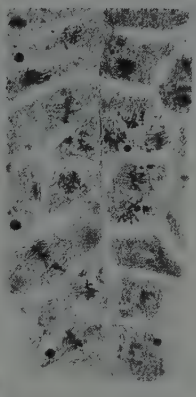

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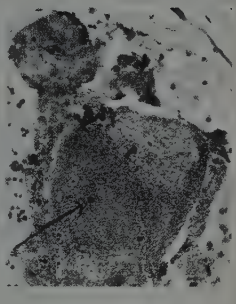

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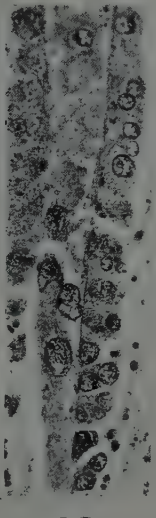

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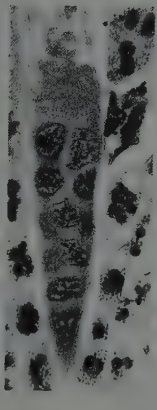

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No. 10.

AN APPARENT CONNECTION BETWEEN THE PRESENCE OF COLLOIDS AND THE OSMOTIC PRESSURES OF CONIFER LEAVES.

By O. ROBERTS, M.Sc., and S. A. STYLES, M.Sc.

[Read February 21. Published separately May 18, 1939.]

During an examination of the pH of conifer leaves it was noticed that, when those of some species were ground with water, considerable quantities of mucilaginous colloids present caused the mixture to become slimy and viscous. Colloids of this type have been instanced as a factor in the water-holding power of the cell against frost and drought, but little attention seems to have been given to the part they may take in the water relations of the cell under normal conditions.

Bennet-Clark and others (1936), working on *Beta, Rheum,* and other plants, have observed differences between the osmotic pressures derived plasmolytically and cryoscopically. These differences they explain by introducing the concept of positive water secretion by the protoplasm into the vacuole. It seems feasible that the water-imbibing power of mucilaginous colloids might also cause differences between plasmolytic and cryoscopic values of osmotic pressure, the plasmolytic method giving higher values, which include the pressures developed by the colloids, which the cryoscopic method does not measure. The osmotic pressures of leaves from species which contained colloids, and from species which were relatively colloid-free, were, therefore, measured by both methods. The results here outlined are only of a preliminary nature, but seem so definite and suggestive as to appear worth recording pending a fuller examination of the whole problem.

Methods.

Only some conifers have leaves which could be used. Those, for example, with cupressoid leaves, with the exception of *Thujopsis,* did not cut into satisfactory sections. Pines also were not used, because of the large area of transfusion tissue.

Material was collected and examined on the same day. Sucrose solutions were used for the plasmolytic method. A range of solutions with osmotic

pressures from 12 to 54 atmospheres was made up from B.D.H. sucrose, and calibrated cryoscopically.

The average osmotic pressure of the leaf was determined by finding the solution, in which 50 per cent. of the cells showed plasmolysis. Sections were cut by hand from leaves from the sample, the remainder of which was pressed, and the osmotic pressure of the expressed sap determined cryoscopically. The sections, one from each leaf, were placed in the series of solutions, and after one hour 100 cells were counted (25 from each section) in each of the series. Using the data thus obtained a graph was plotted, and the osmotic value at which 50 per cent. of the leaf cells would be plasmolysed read off.

The length of time the sections should remain in the sucrose solution was determined by a preliminary experiment with *Taxus baccata* leaves in solutions of different concentrations, giving the following results:—

Immersion time for maximum plasmolysis of *Taxus*.

	% cells plasmolysed after			
	15 mins.	30 mins.	45 mins.	60 mins.
20 % Sucrose	11 %	17 %	23 %	24 %
30 % Sucrose	13 %	23 %	35 %	37 %
40 % Sucrose	19 %	35 %	43 %	44 %
50 % Sucrose	28 %	41 %	55 %	54 %

As the difference between 45 mins. and 1 hour was very slight the latter period was chosen as the standard time of immersion.

Since a large difference in average pressure between the mesophyll and palisade cells would necessitate the relative volumes of the tissues being considered, their respective average pressures were determined, again using *Taxus*.

Osmotic Concentrations in Atmospheres of Palisade and Mesophyll in different samples of *Taxus*.

	I.	II.	III.	IV.	Average.
Palisade ...	39	38	40	37	38·5
Mesophyll ...	37	34·9	36·5	34	35·6

Variations in the volume of the cells, due to shrinkage of the cell wall as the contents of the vacuole come into equilibrium with the plasmolyticum, might be expected to cause the plasmolytic results to be too high, since a certain proportion of the cells would not show plasmolysis, and would be counted as unplasmolysed, even though they would have lost water to the external solution. The cell walls of conifer leaves, however, are relatively thick and

heavy, and it was felt that the shrinkage from the normal volume would not be very great. Some measurements of the cell volumes confirmed this impression. Sections of *Keteleeria, Thuyopsis, Araucaria araucana* and *A. excelsa, Torreya californica, Taxus baccata,* and *Cephalotaxus drupacea,* were allowed to stand in water until the cells were fully turgid. Individual cells were then measured with an ocular micrometer. The sections were plasmolysed and the same cells measured again. As a check on these results the outlines of separate cells of other sections were drawn on white paper with a projector and their outlines compared when turgid and when plasmolysed. The average shrinkage of the leaf cells from full turgidity to plasmolysis varied with the species, but ranged from about 4 per cent. in *Araucaria araucana* to 10 per cent. in *Cephalotaxus.* Since the leaf cells are seldom fully turgid, the shrinkage from the normal to the plasmolysed volume would be even less than this amount. The results presented here are not corrected for the error caused by this decrease in volume, since its effect would be small, and would affect all the plasmolytic results in the same direction.

One other source of error is worth mentioning. The cell walls of some species, such as *Abies firma* and the araucarias, are encrusted with deposits of crystals, presumably calcium oxalate, which make it very difficult to see the protoplasm underneath, so that in counting the cells it is often hard to say whether or not they are plasmolysed.

Cryoscopic measurements of the expressed sap were made with the Beckmann freezing-point apparatus, taking the usual precautions, and using the formulae given in Loomis and Schull (1937). The leaves were killed by Walter's (1931) method, and pressed between plates covered with silver foil. A few preliminary experiments with *Taxus baccata* gave complete agreement whether the leaves were killed by freezing in liquid air or by Walter's method. As the latter was more convenient it was accordingly used. Most of the leaves dealt with consist mainly of palisade and mesophyll cells, so that the error caused by dilution by the sap in the vascular bundles and epidermis is very slight. However, in two species, *Thuyopsis* and *Saxegothaea,* the leaves contain large numbers of colourless cells whose total volume compared with the rest of the leaf cannot be ignored. Due to their lack of chloroplasts it is almost impossible to see incipient plasmolysis, and so to determine their pressure plasmolytically. If they are a form of water-storing tissue with a different osmotic pressure from that of the chlorenchyma, then when the leaves are pressed out their contents will affect the validity of the cryoscopic figure as an expression of the average pressure of these leaves.

The relative mucilaginous-colloid content of the different species was determined by comparing the outflow time at room temperature, through a 15 cm. capillary tube 0·5 mm. internal diameter, of 5 c.c. of water and of 5 c.c. of the various saps, or of filtered suspensions of ground-up leaf material and water.

Results.

The results of the viscosity measurements are presented in Table 1. Water is taken as unity. In the first column are the viscosities of the fluids obtained by grinding up 2 grams of leaves in a mortar as finely as possible, adding 10 c.c. water and filtering. In the second column are the viscosities of the expressed saps. In this case, the saps were expressed from leaves which had been killed by freezing in liquid air.

Table 1.

Relative Viscosity of Leaf Saps.

(Water taken as unity.)

	I.	II.
Taxus baccata (*a*)	1·41	1·66
Cephalotaxus drupacea	1·40	1·50
Torreya californica	1·25	1·58
Keteleeria Davidiana	1·08	1·33
Picea pungens	1·16	1·41
Picea excelsa	1·08	1·33
Cedrus deodara	1·20	——
Sequoia sempervirens	1·86	1·80
Sciadopitys verticillata	1·25	1·50
Podocarpus salignus	1·10	1·25
Taxus baccata (*b*)	7·4	8·0
Abies firma	2·50	5·0
Thuyopsis dolobrata	5·41	6·7
Araucaria araucana	5·0	12·4
Araucaria excelsa	22·4	60·0
Araucaria excelsa var. *varieata* ...	27	60
Saxegothaea conspicua	8·20	——

The first ten species in Table 1 all have saps which are quite fluid and mobile, while the last seven have thicker viscous saps resembling a 4 or 5 per cent. starch solution. The amount of colloid varies in the leaves of different trees of the same species, and in this respect *Taxus baccata* is peculiar, since some trees can be found which contain very little. Two such different specimens of *Taxus baccata* are labelled (*a*) and (*b*) respectively in the Tables 1 and 2.

The osmotic pressures determined plasmolytically and cryoscopically are shown in Table 2. Those species with sap viscosities less than twice that of water are grouped under the heading "Non-colloidal," while those which are mucilaginous are termed "Colloidal."

TABLE 2.

OSMOTIC PRESSURES IN ATMOSPHERES OF LEAF SAP OF CONIFER SPECIES DETERMINED CRYOSCOPICALLY AND PLASMOLYTICALLY.

	Cryoscopic Value.	Plasmolytic Value.	Difference.
NON-COLLOIDAL—			
Taxus baccata (*a*)	21·20	25	3·80
Torreya californica	21·00	23	2·00
Picea pungens	19·74	23	3·26
Picea excelsa	20·64	23·5	2·86
Podocarpus salignus ...	20·40	25·1	4·70
Cedrus deodara	22·55	24·5	1·95
Sequoia sempervirens ...	21·04	22·3	1·26
Keteleeria Davidiana ...	22·70	23·2	0·50
Cephalotaxus drupacea ...	17·90	15·0	2·90
Sciadopitys verticillata ...	16·20	20·0	3·80
COLLOIDAL—			
Taxus baccata (*b*) ...	21·00	33·00	12·00
Abies firma	22·22	28·70	5·48
Araucaria araucana ...	17·00	38·50	21·50
Thujopsis dolabrata ...	14·10	35·00	20·90
Saxegothaea conspicua ...	17·07	28·00	10·93
Araucaria excelsa ...	25·43	38·00	12·57
Araucaria excelsa var. *varieata*	21·30	35·00	13·70

So far as the cryoscopic figures are concerned they agree in general magnitude with those of other workers on the osmotic pressures of conifer leaves, references to which will be found in Walter (1931).

The osmotic pressures of the non-colloidal leaves as determined by the plasmolytic method are in the main higher by one or two atmospheres than the cryoscopic values. Some of this difference may be due to secretion by the protoplasm, as Bennet-Clark suggests, but even closer agreement could be attained if these results were corrected for cell volume change during plasmolysis. On the other hand, the plasmolytic figures are markedly higher in the mucilage-containing species.

It is interesting to compare the two *Taxus* forms (*a*) and (*b*) in the Tables, one of which gave a fluid mobile sap, the other being viscous or colloidal. Both have nearly the same osmotic pressure if determined cryoscopically, but plasmolytically the non-colloidal one is 25 atmospheres, while the colloidal form gives a figure of 33 atmospheres or more.

That this difference persists through the year in the colloid-containing species is shown by Table 3. The cryoscopic values show the usual spring fall from the higher winter values, but the plasmolytic figures, though always greater, do not seem to show corresponding seasonal changes.

TABLE 3.

SEASONAL VALUES IN ATMOSPHERES OF THE OSMOTIC PRESSURE OF LEAF SAP IN *Taxus Baccata* (*b*).

Date.	Cryoscopic Value.	Plasmolytic Value.	Difference.
14/1/37 ...	21·0	33	12·0
3/2/38 ...	20·1	35	14·9
18/2/38 ...	20·0	36	16·0
16/3/38 ...	20·6	33	12·4
1/4/38 ...	18·9	32	13·1
4/4/38 ...	18·0	34	16·0
9/5/38 ...	18·3	34	15·7
13/6/38 ...	17·3	35	17·7
14/7/38 ...	18·7	33	14·3
11/8/38 ...	21·7	34	12·3

The difference between the cryoscopic and plasmolytic figures for *Thuyopsis* and *Saxegothaea* may to some extent be due to the contents of the water cells in these leaves diluting the expressed sap and so lowering the cryoscopic figures. But even if this is the case we do not consider that the volumes of these cells relative to the volumes of the remainder of the leaves are sufficient to account for more than a few atmospheres.

The results for *Taxus, Abies,* and the three araucarias were quite clear, and, compared with those for the non-mucilaginous species, seem to justify the conclusion that the value of the osmotic pressure of colloid-containing conifer leaves measured plasmolytically is always higher than when derived cryoscopically.

It is of interest to note that Oppenheimer's (1932) figures for the osmotic pressure in leaves of a flowering plant, which has a viscous sap, show a difference of 6 atmospheres when measured by both methods, the actual figures being—plasmolytic value, 29; cryoscopic value, 23. Malin (1931) also records a figure of 28 for the suction pressure of *Taxus*. The suction pressure is, of course, less than the osmotic pressure.

It is not easy to account for these differences, as there does not seem to be any direct relationship between viscosity and increased osmotic pressure, but it is unlikely that the colloids are chemically identical in the different species.

That colloids in seeds, etc., can absorb water with tremendous force is well known, as is the fact that dilute solutions of gum arabic and protein have low osmotic pressures, but information is scanty as to the total water-absorbing force, imbibitional and osmotic, of systems such as these saps containing colloids and crystalloids together. Some attempts were made to measure the pressures of the expressed sap of *Taxus* and *Araucaria* osmometrically, but, due to the difficulty of preparing a satisfactory semipermeable membrane, the results were not conclusive. Sections of beet and *Torreya* when placed in *Taxus* sap were killed by it, and consequently could not be used as osmometers,

but some experiments using *Taxus* sections in *Taxus* sap which had been cleared of colloids (by precipitating with alcohol, evaporating the alcohol off at 30° C., and making up to volume with distilled water) gave as an average the following results :—

	Cells Plasmolysed.
Taxus sections in expressed whole sap. Cryoscopic pressure, 21·5 at.	45–50 %
Taxus sections in colloid-free sap. Cryoscopic pressure, 21·0 at.	30–33 %

From these it would seem that the colloid-free sap has a much lower plasmolysing power than when the colloid is present.

Apart from their possible water-retaining powers, these colloids may fulfil another function in the leaf. It has been suggested that the death of leaves on desiccation is due to the mechanical rupture of the protoplasm from the cell wall as the vacuole loses water. It may be that these colloids with their content of imbibed or adsorbed water are capable of losing moisture to some extent without a proportional decrease in volume, and thus prevent the tearing away of the protoplasm from the wall.

While our results for the non-colloidal species agree with those of Bennet-Clark in that the plasmolytic figures are slightly higher than the cryoscopic values, and to about the same extent, our figures for the colloidal species suggest that some other factor than water secretion by the protoplasm is at work. For the time being, it is suggested that this factor is the presence of the mucilaginous colloids in the vacuolar sap. If these results can be shown to be applicable to other plants besides conifers, it would appear that some of the work on osmotic pressures, determined cryoscopically, may need reviewing.

We wish to record our thanks to Professor Doyle, at whose suggestion this work was undertaken, for his frequent assistance and advice; and to Mr. Besant, Curator of the Botanic Gardens, Dublin, for his courtesy in affording us access to the plants used.

SUMMARY.

1. The osmotic pressures of conifer leaves of different species were determined both plasmolytically and cryoscopically.

2. The results by both methods agree well when the leaves do not contain mucilaginous colloids.

3. When such colloids are present the osmotic pressures by the plasmolytic method are much higher than the values derived cryoscopically.

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No. 11.

EMBRYOGENY IN *SAXEGOTHAEA* AND ITS RELATION TO OTHER PODOCARPS.

By J. DOYLE and W. J. LOOBY
(Botany Department, University College, Dublin).

[Read March 21. Published separately May 31, 1939.]

In a recent paper (Looby and Doyle, 1939) an account was given of the development of the gametophytes and of fertilization in *Saxegothaea* up to the early proembryo stage. Here the further history of the embryogeny is considered and certain conclusions drawn.

Embryogeny.

The development was followed out from dissected embryos occasionally supplemented by sections of the ovules. The few cases in which reference is made to these latter are specifically mentioned to distinguish them from the main bulk of the material, which is unspecified and which was obtained by dissection. The embryos, being embedded deep in the developing endosperm, were sometimes difficult to expose and remove, but if the endosperm was opened along its narrow edge the losses were few. The material was derived from the same source as that described in the recent paper, and practically all came from the 1935 collection. In that year, following a free coning in the previous season, an unusual number of developing fertile ovules were available. Owing, however, to the mistaken idea, obtained from an earlier collection, that the fertilization and early proembryo stages were later than they actually proved to be, collections made up to the middle of June were comparatively meagre, and the bulk of the ovules were collected in stages up to mid-July. Most of these were far too late for the critical stages near fertilization, and were thus fortunately available for this embryogeny study.

As there are certain important variations in the course of the development this short account will first refer to what appears to be the normal type, and will then describe the variations.

Normal embryogeny.

In this case cleavage does not occur, but simple polyembryony—the development of embryos from separately fertilized archegonia—is fairly common, although in no case was more than one embryo found in the oldest stages available.

A brief account of the early proembryo has already been given (Looby and Doyle), and need not here be repeated in full. Shortly after fertilization sixteen

free nuclei are found at the base of the long-pointed archegonium. The first appearance of walls was not seen, the earliest stage available, drawn in Fig. 1 A from a section, already showing the prosuspensors *ps* just beginning to elongate. A tier of free nuclei *o* open to the archegonium is left above; elsewhere, in the prosuspensor tier *ps* and the embryonic group *e,* the cells must originally have been uninucleate. At an early stage, however, the nucleus in each of the cells of the embryonic group, but not in the prosuspensors, divides, the divisions being nearly simultaneous. The group of embryonic cells remains in this binucleate condition for some time, during which the prosuspensors undergo rapid elongation, resulting in the stage shown in Fig. 1 B. This stage is still young, as the prosuspensors *ps* are straight and relatively small in comparison with the embryo group *e.* The functioning prosuspensors are commonly four, but three and five have been found. In the latter case one, or even two, may be unable to keep pace with the others, may cease elongation, and tend to collapse. In most cases in sectioned material an open tier of free nuclei—2 to 5 in number—can be seen, but they degenerate early, and are never seen in the dissected embryos. Once only was one of these nuclei seen to be walled—Fig. 2 A *r*. In the figure about half of the prosuspensors *ps* are cut away below, and the cell *r* is accompanied by at least one free nucleus and another nucleus which may possibly be walled also. Occasionally also slight abnormalities appear in the upper region of the prosuspensors. The cells which go to form these may not be regularly arranged in one tier, and two cells end to end may begin to elongate, as is shown in cell *c* in Fig. 2 B. A number of variants of this condition have been seen, in which cells such as *c,* but in a less favourable position, have been thrust aside by the growth of more rapidly elongating cells close by. It is difficult to call a cell like that in Fig. 2 B *c* a "rosette" cell, or even that in Fig. 2 A *r,* if their erratic origin is compared with the development of a clear rosette tier such as is shown in *Sequoia gigantea* (Looby and Doyle, 1937), in which the uppermost tier of embryonic nuclei by a simultaneous division cuts off a group of nuclei which soon become walled to form a very distinct group of rosette cells. There would thus seem to be some need to clarify the conception of the "rosette" tier in certain conifer embryos. Even if the cell in Fig. 2 A *r* be deemed a rosette cell, those like that in Fig. 2 B *c* are clearly of the nature of prosuspensor cells in an early embryo in which the tiering of this layer became a little disorganized.

The embryonic cells remain binucleate until the prosuspensors have become quite long and have begun to bend. Eventually a membrane forms between each of the pairs of nuclei, and further divisions are initiated. At this stage, and for some time afterwards, an apical cell may be distinctly seen in many cases, as in Fig. 1 C *a* (from a section) and 1 F *a*. Secondary suspensors, or embryonal tubes, develop at a comparatively early stage, and are indicated as *et* in all drawings. They form a single belt of elongated tubular cells, which are added to by the growth of other cells between those already extended.

Fig. 1 D shows diagrammatically the typical arrangement at this stage, and Fig. 1 E, F show the embryo with the secondary suspensors drawn more accurately to scale. The existence of the apical cell is of some interest. It

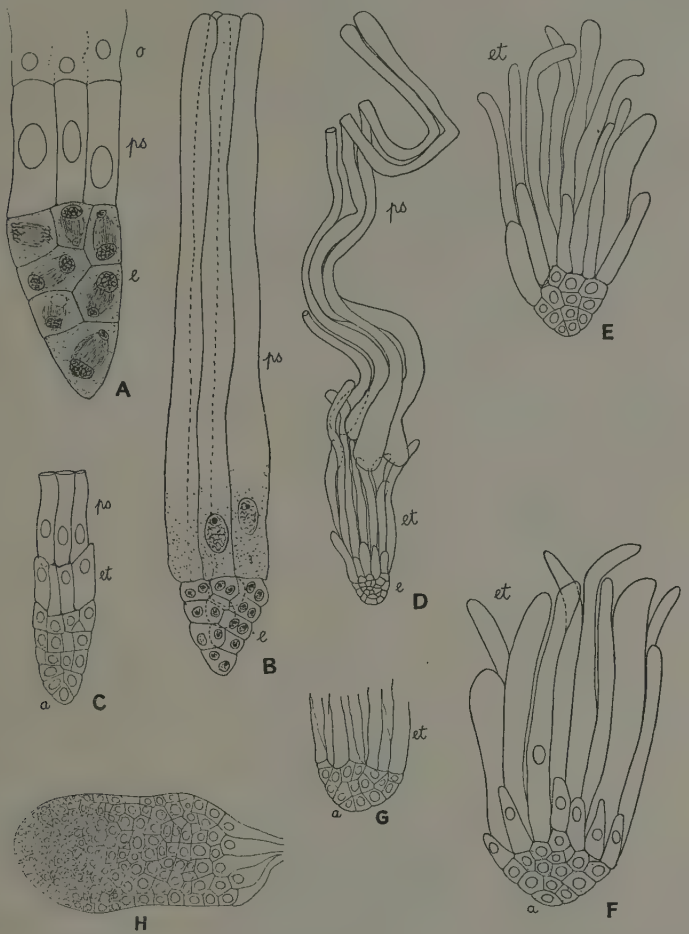

FIG. 1.—*Saxegothaea.* A. Primary embryonic cells becoming binucleate × 300. June 25. B. Typical young proembryo. × 135. July 3. C. Secondary suspensors beginning to elongate. × 135. July 3. D. Typical older stage. × 55. July 8. E, F, G. Embryo cells and secondary suspensors. × 135. July 8. H. Embryo at shedding at seed. × 135. Aug. 10.

Prosuspensors *ps*; secondary suspensors *et*; apical cell *a*.

is recorded for *Dacrydium* (Buchholz, 1933) and *Phyllocladus* (Holloway, 1937), but not for *Podocarpus*, at least as far as the *Stachycarpus* section is concerned. In the two species of *Podocarpus* in this section—*P. spicatus* and *P. ferrugineus*—examined by Sinnott (1913), and later by Buchholz (1936), a

very distinct terminal cell is seen at the binucleate stage. In *P. spicatus* this functions somewhat like the cap cells in *Cephalotaxus*, and is crushed away together, sometimes, with a few neighbouring embryonic cells, while in *P. ferrugineus* the terminal cell is never lost in the same way, but becomes part of the embryonic group which shows no distinct apical cell at any of the subsequent stages. In *Saxegothaea* an apical cell is usually quite definite for some time. It is distinct in the section from which Fig. 1 C was drawn, while in the dissected embryo, Fig. 1 F, the apical cell was as distinct as it commonly is in *Dictyota*. This is admittedly an extreme case of definiteness, but in many other examples there was no doubt as to its presence. In *Saxegothaea*, however, the embryo has to advance through a compact cone of starch containing cells—*c* in Fig. 2 C—and frequently there is difficulty in this advance. Embryos have been found, both in section and in the whole, which were unable to make headway and remained in the same position with the prosuspensors coiled around them, or which had taken a very oblique and usually quite unsuccessful course. Even normal embryos, in dissection, were commonly found tightly pressed in against the cavity in the starch cone. They could seldom be removed by gentle tension on the suspensors, but actually had to be dissected out with fine needles, usually bringing with them some of the starch cells. In some such cases a few crumpled cells were to be seen at the tip, but it was uncertain if these were really cells of the embryo or of the starch cone from which the starch grains had been digested. In embryos such as those shown in Fig. 1 E and Fig. 1 G (the latter from a section) in which an apical cell cannot be determined with certainty, it is thus not clear whether it was crushed during growth or merely, at a very early stage, became one with the other cells of the embryo. In most of these cases, however, it was considered that, in the strongest probability, there was no crushing, the apical cell simply losing its definiteness at a very early stage. Thus, in *Saxegothaea* there may be an apical cell of some duration, or it may disappear as such at a very early stage. The early disappearance of the terminal cell in *Podocarpus spicatus* may be related, not to the organization of any special embryonic cap region, but to the difficulty of penetrating the corresponding starch cone which apparently also occurs in this species, *P. ferrugineus* resembling those cases in *Saxegothaea* in which the functioning of a special apical cell is not retained.

Growth is comparatively slow up to mid-July, most embryos showing the stages figured in Fig. D, E, F, or perhaps a little more advanced, but in all cases with an embryo group still comparatively small. Unfortunately a gap in the collecting occurred at this time, the next date available being the collection of August 10. The embryo, Fig. 1 H, has almost reached the stage of the organization of members, the prosuspensors and most of the secondary suspensor mass being now crushed and flattened by the pressure of the surrounding endosperm cells. About this date in the trees at our disposal at Kilmacurragh, Co. Wicklow, the seeds are shed from the cones or the cones themselves drop off with the contained seeds. It is not known if this occurs in the plant's

natural habitat; nor has it been possible to determine how far embryo development proceeds after shedding, or along what lines, since in no recent year have more than a few cones been left on the trees till this shedding period, and any old cones on the ground had rotted. It is, however, definitely of interest that, in the *Stachycarpus* section of *Podocarpus,* and probably also in other podocarps, a similar phenomenon seems to take place (Buchholz, 1936).

Certain features in the development of the maturing endosperm are shown in Fig. 2 C, D. Fig. 2 C is a composite diagram, which includes a number of stages which tend to overlap. After fertilization the long archegonial cone *a* remains for some time after the embryo *e* has begun to elongate. The cells of the upper part of the prothallus close over the tops of the archegonia, and subsequently become large, vacuolate, and tenuous, forming a cap of delicate tissue *o*. Below the archegonia a zone of cells *c,* with numerous starch grains, becomes increasingly prominent—the starch cone—into which the embryo bores, and out of which it must frequently be gently dissected. This starch continues up along the sides of the archegonial region as a zone of cells *i,* intermediate between the jacket cells and the main cells of the prothallus. As the structure ages the whole zones *a* and *o,* with a varying amount of the inner zone of *i,* gradually collapse, the cavity being closed by the inward expansion of the outer prothallial

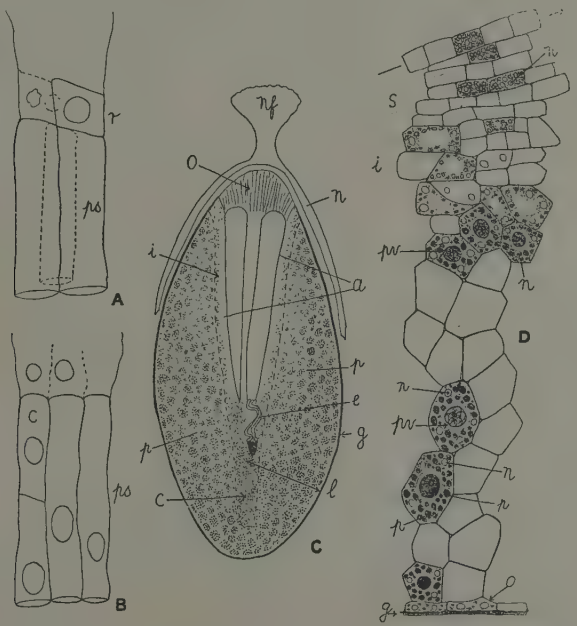

FIG. 2.—*Saxegothaea.* A, B. Upper part of two young proembryos. × 300. June 24, 19. C. Diagram to show plan of ovular development to seed. × 20. D. Structure of developing endosperm. × 135. July 8. Explanation in the text.

cells. There is left, however, at least up to the time of shedding, a flattened slit leading down to the position of the growing embryo. The presence of this slit helps considerably in the opening of the young seed for the removal of the embryo. It is shown in a drawing in a short note by Saxton (1929) on the older fertile ovule of *Saxegothaea* (his Fig. 2, p. 376). The nucellus *n* becomes reduced to a thin papery membrane except towards the top where the nucellar flare *nf* is still prominent, plugging an opening in the thickened integument like a stopper in a water-bottle. The arrangement of flare and integument is nicely shown in Fig. 1 of Saxton's note.

The structure of the endosperm tissue is shown in Fig. 2 D, which is a drawing of the cells along the two-headed arrow line *l*, in Fig. 2 C. In Fig. 2 D the arrow line shows the position and direction of the middle line of the ovule, which here coincides with the middle line of the cone of starch cells *s*. These are small and rectangular with no obvious vacuole. To the outer side they pass into an intermediate zone *i* of vacuolated and somewhat irregular cells, which pass rather abruptly into the main mass of the endosperm. The cells of this tissue are large, and become densely packed with protein grains of some size. Most references to food reserves in podocarpean endosperm mention starch as the storage material, although no indication can be found of any microchemical tests having been applied. Here, however, a section stained with iodine shows very clearly an inner blue zone and a large outer yellowish-brown one. This protein storage is peculiar, and would merit more detailed investigation. The protoplasm, at a younger stage very thin and surrounding a wide vacuole, becomes much thicker after fertilization, and the vacuole correspondingly smaller. This vacuole is now occupied by a single large protein body *pv* showing a structure which has not yet been investigated. In the protoplasm other smaller protein grains *p* are deposited which show a structure like the better-known aleurone bodies in angiosperm seeds; they are much more numerous than in the figure. This clumping of protein grains in the cells is roughly shown in Fig. 2 C, which gives the general appearance as seen under low magnifications. In this figure also the radial arrangement of the original tissue, as derived from the alveoli, is lightly indicated at places. All these prothallial cells contain three to five nuclei *n*, which, especially in the protein zone, are difficult to see, being obscured by the storage grains. The intermediate cells contain some protein grains as well as starch. The gynospore membrane *g* remains as a thick double layer up to the shedding period, and immediately within it lies the outermost layer of the endosperm *o*, a single layer of narrow rectangular cells with two or three nuclei and little protein. The protein accumulation gradually encroaches on the starch core especially towards the base, but at the time of shedding this core is still quite distinct.

How far this general structure is characteristic of the endosperm of other podocarps is not clear. The single outer layer of small cells seems, however, fairly common in *Podocarpus* (Coker, Gibbs 1912), although the suggestion of

Coker, that it is a functional layer replacing the tapetum in forms in which this latter is absent, must be disregarded. There are also indications in *Podocarpus* and *Dacrydium* of a definite subarchegonial cone and an outer storage area (Sinnott, Gibbs Fig. 39), but details are lacking. A single outer layer seems also to occur in *Phyllocladus alpinus,* from which species Holloway also draws a few endosperm cells, with (in the central vacuole) a large body, which, although labelled an oil-drop, may well be protein. A fuller microscopical and biochemical investigation might well yield fruitful results.

Variations in embryogeny; polyembryony.

Various types of lobed embryos, embryos showing budding, and even occasionally double embryos, were found, which are illustrated in Fig. 3. A fairly advanced double embryo is shown diagrammatically in Fig. 3 A, the embryo group proper and the secondary suspensors being drawn in Fig. 3 B. It can be seen that the prosuspensors *ps* are being pushed up by a mass of secondary suspensors *et* which carry the double embryo below. The two embryo clumps are not completely separated, but have a common basal mass of irregular tissue, and are quite definitely attached to a common group of prosuspensors. Both embryo clumps are probably controlled by an apical cell. No later stage than this was seen, nor was any trace of this embryo splitting found in the final collection in August. If material had been available from mid-July onwards the subsequent developments, if indeed such embryos survive for any length of time, might have been followed. It is shown, however, in Fig. 3 B that the embryonic clump on the left has begun to develop additional secondary suspensors e^2, further active formation of which could gradually separate this zone from the smaller clump on the right. It is possible that some such development as this may explain the single example of apparent polyembryony described by Buchholz (1936) in *Podocarpus ferrugineus* (his Fig. 15). A younger but similar stage is shown in Fig. 3 C from a section. Fig. 3 D shows an example of more simple lobing. The secondary suspensors *et* carry an embryo clump—drawn in continuous lines—which is of a normal type except that it carries at one side a projecting lobe—shown with broken lines—which is quite small but possesses a very definite apical cell. Fig. 3 F shows a young stage, from which the stage in Fig. 3 B can be derived. Two of the prosuspensors *ps* are drawn to indicate that the secondary suspensors *et* are only starting to elongate. It is clear if development proceeds along the lines indicated that a common mass of secondary suspensors will ultimately carry a bilobed embryo, each lobe with an apical cell. It is possible that a third small lobe has been formed—the small dotted area—but it proved difficult to decide on its nature and extent in this case. However Fig. 3 E shows an example with two main lobes joined by a common mass of cells, from which a common group of secondary suspensors is arising, and to which a small third lobe is attached somewhat as in the case of Fig. 3 D. These lobed embryos were found

in about 15 per cent. of cases. Few of them, however, seem able to continue development, since, although fairly frequent in the early July collections, only two—and these of the more regular advanced type of Fig. 3 A, B—were found in the collection of July 16.

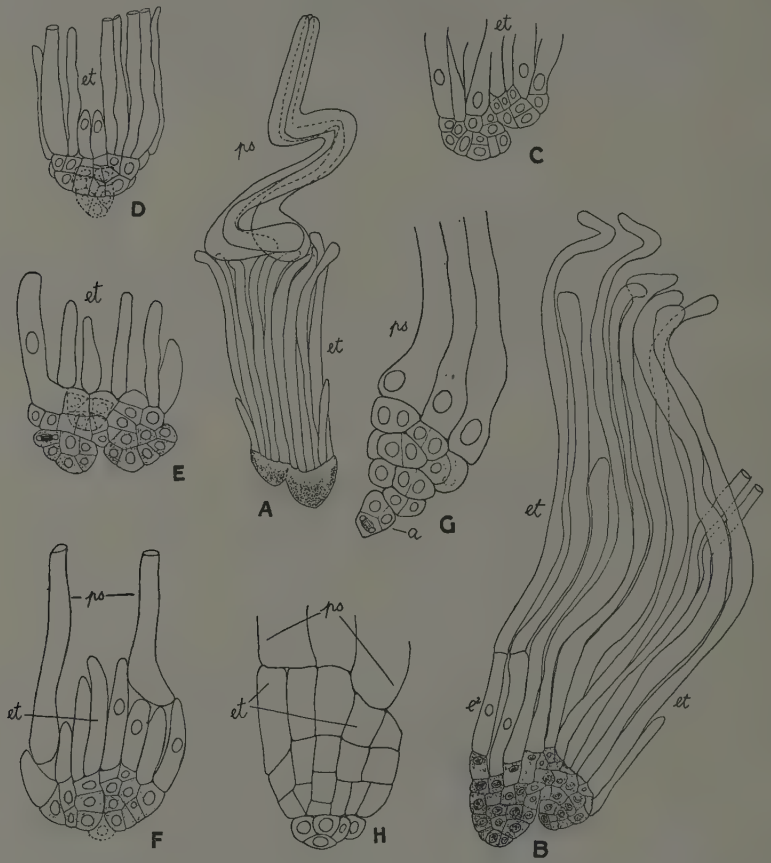

FIG. 3.—*Saxegothaea.* A. Complete double embryo. × 55. July 16. B. Secondary suspensors and embryonic cells of same. × 135. July 16. C. Younger stage of B from a section. × 135. July 8. D, E, F. Lobed embryos. × 135. July 8. G. Origin of lobing. × 135. July 3. H. Young stage with apical lobe only and secondary suspensors beginning to elongate. × 250. July 3.

The origin of this lobing is clear enough. The embryonic cells in the young proembryo remain binucleate for some time, but, shortly before the plate is formed which initiates the uninucleate stage, the cells increase in volume. This increase in volume in a group of cells which have not yet formed a true tissue seems to result, frequently if not always, in a slight rounding-off and

a partial separation of these cells. It is well known in the segmentation of the fertilized animal egg that the early cell group can be separated in some cases into single cells, each of which may develop separately. The embryonic cells in *Saxegothaea* seem in the same way to be all potential embryo initials, as Buchholz has argued from other data, and some, at the critical stage of this cell-plate formation, may start divisions independently of the others. This stage is shown in Fig. 3 G, in which an apical group of cells *a* has been formed, and in which the tendency towards separate rounding-off is shown by all the cells. Whether or not due to the proximity and control of the pro-suspensors the cells in their neighbourhood remain intact, so that even if a number of lobes—derived from the independent divisions of the other cells—are formed they remain grouped together and carried by a common mass of secondary suspensors. Fig. 3 H shows the further development of a simple case in which the apex only was involved, as in Fig. 3 G. The secondary suspensors *et* just show signs of elongation, and the majority of the embryonic cells have further divided to form a uniform tissue, leaving the apical region as a special lobe. Development of lateral lobes proceeds in the same way. It is not easy to place this indefinite lobing of the embryo group of cells in *Saxegothaea* under any of the recognized forms of polyembryony, but it has the peculiar interest that it can lead directly to that form of polyembryony called "determinate cleavage," and described by Buchholz (1929, 1933) for *Dacrydium cupressinum*. Here each of the binucleate cells of the proembryo—5 to 7 in number—gives rise to a group of cells, and each group may develop into an embryo. Usually, however, the successful functioning embryo comes from the apical group. Any embryo group which develops further forms its own secondary suspensors, thus giving rise to a true type of cleavage polyembryony.

DISCUSSION.

Comparisons were made, in the recent paper already referred to (Looby and Doyle), between the gametophyte stages in *Saxegothaea* and those in other podocarps; and from these there appears to be a remarkable similarity in the male and female gametophytes of *Saxegothaea, Dacrydium, Phyllocladus,* and the *Stachycarpus* section of *Podocarpus*. The presence in the pollen grain of two prothallial cells of which at least one further divides, the liberation of prothallial nuclei free in the protoplasm of the grain, the lateral derivation of the stalk cell (or nucleus) from the generative one, and the lagging of the body-cell in the germinating grain, are common to all. The female gametophytes show the following points in common:—(*a*) origin from a single large gynospore mother cell surrounded by a tapetum; (*b*) frequent germination of two spores; (*c*) a thick two-layered membrane; (*d*) few—2 to 4—long and narrow-pointed archegonia whose egg nucleus, of relatively large size, is fertilized at the top of the egg (*Phyllocladus* may possess archegonia, of the

same plan, but not quite so long as the others); (*e*) archegonial neck of one tier; (*f*) the suspension of the neck cells across the upper part of the archegonium by a membrane; (*g*) the large pit above the neck cells which is occupied by the pollen tube. Until cogent evidence is offered to the contrary it may then be safely taken that these structures were, in plan, characteristic of the primitive podocarpean stock, since they appear in forms so divergent in many other respects. *Saxegothaea,* showing as it does in addition to a compact cone a further primitive feature in the possession of two equal or sub-equal male cells, would appear to be, among living podocarps, the nearest to the basal type. The method of pollination and the structure of its nucellus are, however, very definitely specialized, and will be referred to elsewhere. On the basis of gametophyte structure the primitive podocarp type would appear to have given rise to the *Phyllocladus* line, the *Saxegothaea-Dacrydium* line, and the *Podocarpus* line. In the higher members of the *Podocarpus* genus the female gametophyte became modified. In some species the gynospore membrane became very thin; in most the archegonia became smaller, oval in shape and more numerous, the mature egg nucleus taking its place in the middle of the archegonium; and in many the neck cells formed several tiers. *Microcachrys* may well be a special genus related primarily to the basal stock close to *Saxegothaea.* Its cone and cone-scales are more similar to this latter genus than to any other podocarp; it possesses a podocarpean pollen grain and a thick gynospore membrane, and above all two equal male cells. Its archegonia, however, form an apical complex, an arrangement which may parallel in the podocarps the similar development in the taxodinean and cupressinean lines. *Pherosphaera* seems to show no podocarpean characters, and if a podocarp at all, would appear to be a very aberrant one. On the basis of gametophytic structure nothing can be said about *Acmopyle.*

It is of obvious interest to see how the facts of embryogeny bear on this. A survey of available data, while showing the enormous gaps in our knowledge, yet permits a tentative picture being drawn when the facts given for *Saxegothaea* are also considered.

Data are given for *Podocarpus* by Coker, Stiles (1912), Sinnott, and Buchholz (1929, 1936). In the *Stachycarpus* section, as shown for *P. spicatus* and *P. ferrugineus,* the fusion of the male and female nuclei is followed by two divisions in the upper part of the archegonium, the four nuclei passing to its base, where sixteen free nuclei are formed. Subsequently walls are formed, and the proembryo shows a group of binucleate embryo cells, a tier of prosuspensors with some indefinite "rosette" cells at their top, and a number of free nuclei above. The actual total number of nuclei and of cells in any one proembryo is not distinctly mentioned, but, since *P. spicatus* may have 7–9 prosuspensors, 9–12 binucleate cells in the embryo group, perhaps 5 walled rosette cells, and a number of free nuclei (see Buchholz' Fig. 2, 1936), it is clear that the number is considerably in excess of 16. It is thus uncertain at what stage walls are formed; if at the 16-nucleate stage, then further erratic divisions

to an unknown extent took place in the cells before the tiered organization of the proembryo, which would seem unlikely. It is possible that a further division at the 16-nucleate stage gave rise to a 32-nucleate stage. Such a nuclear division should probably be a simultaneous one, as it is unlikely that erratic divisions would occur in some of the nuclei in this free nuclear condition. In *P. ferrugineus* it is possible that wall formation took place at the 16-nucleate stage, as the proembryo may have 6–10 binucleate embryonic cells and 6 or fewer prosuspensors. *P. usambarensis* would appear to have formed a 32-nucleate proembryo, as a binucleate group of about 9 embryonic cells is described with 20 prosuspensors or more and some rosette cells and free nuclei. The origin of the binucleate condition of the embryonic cells is, however, nowhere described in connection with these species, but in view of the distinct nature of its origin in *Saxegothaea* (Fig. 1 A) it is here assumed that in *Podocarpus* the condition is arrived at in the same manner. As development proceeds the prosuspensors become excessively long, and the whole group of embryonic cells forms a single embryo—there is thus no polyembryony. No clear details are given concerning the development of the secondary suspensor. From Buchholz' (1936) Fig. 15 it would appear to develop late as a stout multicellular compact structure.

Normal embryogeny in *Saxegothaea* resembles in general this development in the *Stachycarpus* species. After fertilization two divisions at the top of a similar long archegonium give four nuclei, which, passing to the base, yield a 16-nucleate stage followed by wall formation, the proembryo becoming organized as already described. No polyembryony occurs, only a single embryo being formed. The differences would seem to be—(*a*) the possible development in some *Stachycarpus* spp. of a 32-nucleate proembryo stage, and (*b*) less intense elongation in *Saxegothaea* of the prosuspensors, and earlier development of a secondary suspensor of long tubular cells.

The *Eupodocarpus* spp. are very different. The fertilized egg nucleus only divides once at the top of the archegonium, the two nuclei passing to the base, where at least 16 free nuclei are formed. Here also there is uncertainty concerning wall formation. In *P. coriaceus,* according to Coker, a single binucleate terminal embryonic cell is carried by a group of 14 prosuspensors with 14 free nuclei in a tier above. The origin of the binucleated condition of the single terminal was not determined, but the whole arrangement may be due to the derivation of the free tier by division from the tier which now functions as the prosuspensor, or by derivation without division from a 32-nucleate stage. It is suggested at least that, if a full series were available, the binucleate condition of the terminal cell would be shown to have come from nuclear division in an originally uninucleate cell. This single embryonic cell is, however, characteristic. It may divide to give a single embryonic mass from the upper part of which a secondary suspensor very similar to that of *Saxegothaea* is early formed. It may, on the other hand, divide by vertical walls into four cells, from each of which a single primary suspensor carrying

one embryo cell is formed—a very definite type of cleavage embryony, and, if Coker's suggestion of the derivation of the "rosette" nuclei from the prosuspensor nuclei be correct, a very clean-cut and specialized type of embryogeny in general. In *P. macrophyllus* a single embryonic cell is formed (Stiles), but further details are not available, except that cleavage does not seem to occur. *P. totara,* its variety (?) *Hallii,* and *P. nivalis* form, from the 16-nucleate stage (Sinnott), a proembryo with again the single binucleate embryonic cell, 7–9 prosuspensors and some "rosette" nuclei. Cleavage may occur occasionally, but the details are not available, though "embryos on single suspensors are mentioned," suggesting that the behaviour of *P. coriaceus* is repeated. *P. dacrydiodes,* in the *Dacrycarpus* section, is said to be similar. On the other hand, Buchholz (1929) says that "the terminal binucleate *cells* are increased in number during the period when the prosuspensor cells elongate" in *P. totara* and *P. dacrydioides,* and he also thinks the further development, when investigated, will resemble that in *Dacrydium.* It is here uncertain whether the statement really means that there is more than one primary embryo cell, which can hardly be suggested in view of Sinnott's photograph (his Pl. 8, 32); neither is it clear if the increased numbers retain the binucleate condition; nor can a *Dacrydium*-type of later development be reconciled with Sinnott's mention of the occurrence of embryos on single suspensors. It is thus obvious that within *Podocarpus* many discrepancies and details need clarifying; but it is quite definite that, in addition to the *Stachycarpus* type of embryogeny which that of *Saxegothaea* resembles, there is the type shown by the higher podocarps, with probable variations on it, a type with a single binucleate embryonic cell in the proembryo which may show definite cleavage embryony.

As far as *Phyllocladus* is concerned, nothing is known of its early proembryo except that fertilization takes place at the upper wide part of the archegonium, and that at least eight free nuclei are formed at its base before wall formation (Kildahl, 1908; Young, 1910; Holloway). Buchholz (1929), however, describes the proembryo at the stage of suspensor elongation. His figures 20 A–C are remarkably similar, even in relative proportions and details, to the corresponding stages in *Saxegothaea*—6–8 binucleate embryonic cells with a definite terminal one, 4–5 prosuspensors, a few elongating "rosette" cells at their top, and some free nuclei above. This almost certainly means that wall formation took place shortly after the appearance of the free 16-nucleate stage. No cleavage occurs, the whole binucleate group giving the embryo, and an apical cell is to be seen in the embryonic clump at least up to a 60-cell stage or more. The prosuspensors are not as long as in *Podocarpus,* but no mention is made of the development, if any, of a secondary suspensor stage. At the shedding of the seed the embryo is more advanced than that of *Saxegothaea,* as the stem tip and cotyledons are already determined, although the embryo develops further after shedding. Apart, however, from the lack of information concerning the secondary suspensor, the embryogenies of *Saxegothaea* and *Phyllocladus* are remarkably similar for two genera so different in cone morphology and habit.

Dacrydium presents many difficulties. Fertilization and its immediate results are unknown, but, in view of the shape of the archegonium and the position of the egg nucleus, fertilization probably follows the lines of *Saxegothaea* and *Stachycarpus*. Sinnott merely states that the proembryo develops as in *Eupodocarpus*, while Buchholz (1933), who had the opportunity apparently of consulting the earlier preparations, says that the proembryo is unknown. The earliest definite stages are those figured by Buchholz (1933), and show the arrangement of the *Stachycarpus-Saxegothaea* type of podocarp proembryo when the prosuspensors are elongating. The embryonic cells—6 to 9 in number—are binucleate, and are carried by a prosuspensor group of 7–9; rosette cells or nuclei were not seen. The whole structure strongly suggests a derivation from a younger stage, like that of *Saxegothaea* in Fig. 1 A, but with fewer embryonic cells and more prosuspensors; and until some evidence is available, either in *Dacrydium* or *Eupodocarpus*, of division in the binucleate embryonic cells with retention of the binucleate condition, it is here taken that the group of binucleated cells dates from first wall formation as in *Saxegothaea*. Subsequent development has been clearly followed by Buchholz (1933). It results in a type of polyembryony to which reference has already been made, and which he has termed determinate cleavage. Each of the binucleate embryonic cells divides to give a special group of cells, an embryonic unit, and each unit can develop into an embryo, although normally it is only the terminal unit which becomes successful. In connection with each embryo unit a secondary suspensor is formed composed of long tubular cells, very similar to those described in *Saxegothaea*, and an embryo unit may retain an apical cell up to the 80-cell stage. It should be stated here that when the lobed embryo clumps were first seen in *Saxegothaea* it was thought that determinate cleavage would be characteristic of it also, but in spite of continued diligent search only the type of lobings already described and shown in Fig. 3 could be found; the search was not abandoned until it was reasonably certain that true determinate cleavage did not occur.

From this survey a number of suggestive conclusions may be drawn. Buchholz (1929) claimed that the binucleate condition of the early embryonic cells constitutes a distinctive diagnostic feature for podocarps. The addition of the primitive *Saxegothaea* to the list seems to mean that this claim is completely sound, and that this feature must be regarded as an important criterion of affinity. The further similarity in embryogeny shown by *Phyllocladus* to *Saxegothaea* and the lower *Podocarpus* spp., in addition to the similar features shown by the gametophytes, means that there can be no further doubt concerning its affinities—*Phyllocladus* is definitely a podocarp. Indeed these general similarities are so close that its separation from *Saxegothaea, Dacrydium*, and the lower *Podocarpus* spp., into a special sub-Family (Pilger, 1926) seems doubtfully necessary. Further, although in this *Phyllocladus-Stachycarpus-Saxegothaea* group there are variations in the embryogeny—notably perhaps in the nature of the secondary suspensors and

the possible development of a 32-nucleate stage in some *Stachycarpus* spp.—yet common to all is the development of a single embryo from the primitive embryonic mass. In view of the further fact that two distinct types of polyembryony are shown in two different lines—viz., *Dacrydium* and the *Eupodocarpus* spp.—it seems simplest on the evidence to consider that the organization of the whole embryonic group of cells into one embryo was a character of the primitive podocarpean complex, which has been retained in three different lines, but which has undergone two polyembryonic modifications. The development of the determinate cleavage in *Dacrydium* from the *Saxegothaea* type of embryogeny can be easily understood. It seems quite clear from *Saxegothaea* that the change in the embryonic cells from the binucleate condition is a critical stage. The increase in volume of the cells in an undifferentiated tissue can result, as already suggested, in a partial separation of the cells, some of which may begin to divide independently of the others. Buchholz (1929, 1938) would seem to be obsessed with the idea that every cell of the embryonic group is an actual embryo initial. Rather are they to be considered as potential embryo initials. The cells resulting from the division of the animal egg may be separated by various agencies, and each may give rise to a complete embryo, but there is no question here but that all the cells resulting from the egg cleavage normally form a single embryo, and are destined so to do, in spite of the fact that they occasionally separate and show the potential embryonic tendency of each cell in the undifferentiated mass. This is the view which is here taken of the embryonic group in the simpler podocarps. Here it is considered that this erratic division in *Saxegothaea* of some of the cells in occasional embryos has become a specialized and advanced development in *Dacrydium*. The determinate nature of the terminal group of cells is to be expected from the greater definiteness of the apical cell of the primary embryonic mass, this definiteness being in its turn determined at least partly by the peculiar pointed end of the long archegonium in which the primary cell formation takes place. The nature of the secondary suspensors derived from the embryo units may indicate a further resemblance to *Saxegothaea*. It is considered that *Dacrydium* is related closely to the basal type, from which *Saxegothaea* also took its origin, a view completely in accord with the close similarity in the gametophytes. It would be of great interest to determine whether any such indefinite lobing as is shown by *Saxegothaea* occurs in *Phyllocladus, Dacrydium Franklinii,* and *Podocarpus andinus.*

The cleavage polyembryony in *Eupodocarpus* raises another point. The large genus *Podocarpus* as a whole seems to be a specialized one, and shows within it definite modifications in gametophytes and embryogeny to which reference has already been made. Buchholz (1936) has discussed some interesting points in connection with the fairly advanced *Stachycarpus* species, *P. usambarensis,* whose embryo differs from *P. spicatus* and *P. ferrugineus* in the larger number of prosuspensors—20 or more—and in the arrangement of the embryonic cells in one tier only. Although he accords a more advanced

status on general grounds to *P. usambarensis,* he suggests, since its archegonia are smaller, more oval than those of the two other species, and thus more like the usual conifer archegonium, that this type of archegonium with the accompanying type of proembryo is more primitive than the tiered and pointed proembryo of *P. spicatus* and *P. ferrugineus,* which is related to the more specialized narrow-pointed archegonium which they possess. The evidence, however, points the other way. *Saxegothaea, Phyllocladus alpinus, Dacrydium cupressinum,* and at least three *Stachycarpus* species, including the probably most primitive species *P. andinus,* all possess the narrow-pointed archegonium. Details are not available for *P. andinus,* but in all the others a tiered proembryo is found. It seems, therefore, that even in *Stachycarpus* the primitive long archegonia have become modified into the more general oval form. The condensation of nuclei in the narrow-pointed end which produced the tiered and pointed embryo has been replaced by a wider structure, in which the nuclei can spread more evenly; and it would seem possible that the large terminal cell of the embryonic group which is to be found in all the tiered embryos, but particularly in the *Stachycarpus* spp., *P. spicatus,* and *P. ferrugineus,* has been retained as the single embryonic cell in *Eupodocarpus*; but the detailed relations must remain obscure until the whole genus *Podocarpus* has been properly surveyed.

Buchholz (1929) also, considering the loss of the terminal cell and sometimes of other cells in *P. spicatus,* uses this fact as an argument in favour of the conception that the primary embryonic mass is a group of embryo initials, of which some may be lost without affecting the status of the others, because, if the primary embryonic group is considered a whole unit, it would mean that the stem tip is endogenous. This view seems to be one limited by purely static morphological conceptions. Actually it would appear that the embryonic group is what it appears to be, an undifferentiated mass of cells, from which, after it has developed further, the parts of the mature embryo will be formed; and if a few cells of the primary mass are injured the regenerative power of a group of *living* cells can surely restore the original organization, whatever it may have been. However, it is not proposed here to enter into the general questions arising from a consideration of conifer embryogeny, but one further reference may be made which is suggested by this examination of the podocarps. Buchholz (1929 and elsewhere) maintains that polyembryony is primitive, and simple embryony derived. In podocarps, however, the simplest interpretation appears to be that the type of simple embryony shown, with minor modifications in *Stachycarpus, Saxegothaea,* and *Phyllocladus,* is basal for the group, and that from it two different types of polyembryony have been derived. If polyembryony is derivative here it is probably derivative elsewhere, especially as Buchholz' contention is based on an assumption which is later used as an axiom. He argues that, if the simple embryo were primitive, and if from it by cleavage—as in *Pinus* (1929, Fig. 2, p. 366)—embryos with single suspensors were formed, and if these two types were in competition in one

ovule, then the simple uncleaved embryo would have *obviously* a manifold supremacy from the beginning. The type with cleavage could never survive to perpetuate the mutation, and thus it must be considered that the more efficient type, the simple embryo, has been derived from the less efficient type, the embryo with cleavage. It is this primary premiss which is mere assumption. Other arguments based on the changes in the male gametophytes and the embryo from heterosporous ferns to conifers are difficult to follow without some common agreement as to the nature of the heterosporous "ferns" which preceded the Palaeozoic conifers.

The inherent unity of *Phyllocladus, Saxegothaea, Dacrydium,* and *Stachycarpus,* as shown by their gametophytes and embryos, is further reflected in their physiology. It has been recently shown (Roberts and Doyle, 1938) that the pH of the leaf sap of conifer genera of recognized affinities lies, in general, within definite restricted ranges. Thus *Dacrydium Franklinii, Saxegothaea, Phyllocladus trichomanoides,* and *Podocarpus andinus,* all lay within the range pH 3·9–4·25. The higher *Podocarpus* spp. on the other hand ranged from *P. elatus,* pH 4·5, to *P. Henckelii,* pH 5·75—thus showing a much greater variation within the one genus than in the whole of the other group, and also covering a different and a less acid range. This is fully in agreement with the modifications in gametophytes and embryogeny shown by the higher *Podocarpus* spp.

There are still three genera remaining, *Acmopyle, Microcachrys,* and *Pherosphaera.* Nothing is known of the life-history of *Acmopyle,* and so, on the basis of developmental stages, it need not be here considered, although it is obviously a podocarp. The position of *Pherosphaera* is doubtful. It has no prothallial cells in its winged grains, and no epimatium to the ovule, two of the most characteristic podocarpean features, while, however, possessing podocarpean nodules on its roots (Saxton, 1930). Knowledge of its embryogeny is limited to the existence of a few prosuspensors carrying a small embryonic group of cells, but it is not known if these cells pass through a binucleate phase (Lawson, 1923 b). The female gametophyte, provided with a delicate membrane, forms a number of small archegonia obliquely and deeply placed along the sides. This condition somewhat resembles that shown by some of the *Eupodocarpus* spp., and suggests the possibility that *Pherosphaera* may be an extreme development, parallel to these species. It can hardly be a primitive genus, as some writers claim from the nature of its cone, since both its gametophytes have advanced so markedly from the type which, from the considerations outlined on the previous pages, seems to be the typical podocarpean one. On this basis the cone is one showing maximum reduction from the original form. Provisionally it may be looked upon, perhaps, as an advanced derivative from the *Phyllocladus* line; but, in spite of the root nodules, its permanent inclusion in the podocarps must await demonstration of a binucleate phase in the primary embryonic cells. Florin (1931) has shown also that in certain features of the leaf epidermis it differs from all other podocarps, although some of these features are shared by *Microcachrys.*

This latter genus is also difficult to place. Its pollen grain is typically podocarpean, its cone scales resemble those of *Saxegothaea,* and it possesses two equal male cells: but the body cell does not linger in the tube, and, above all, it develops an archegonial complex like a cupressinean conifer (Lawson, 1923 a). Unfortunately its embryogeny is no better known than that of *Pherosphaera.* In connection with the archegonial complex Schnarf (1933), recalling an earlier statement of Thomson (1909) that the archegonial initials were seen to be widely separated, wonders if any mistake could have been made over Lawson's material. Admittedly Thomson's material showing stages was limited, but on the other hand *Microcachrys,* endemic in Tasmania, rather resembles another endemic Tasmanian form, *Diselma,* and both grow at good elevations in the same Western Range. If any mistake were made it would have been made with *Diselma.* Nothing is known of the development in this genus, but, since it is definitely cupressinean, it would be expected to show an apical complex and two equal male cells; and actually Lawson's drawings of these, although rather diagrammatic, readily suggest the cupressinean type. Until further material is available, this doubt must remain, but for the present Lawson's account can be accepted since, although *Microcachrys* from its cone alone must be a podocarp, yet it shows two other peculiar features. The pH of its leaf sap is not within the *Saxegothaea-Dacrydium* range but within the range of the specialized *Podocarpus* spp., i.e., 5·4 (Roberts and Doyle); and its stomata differ from other podocarps except *Pherosphaera,* in being epistomatic and monocyclic (Florin, 1931). Provisionally, therefore, it may be considered the living representative of a special line taking its origin close to the stock from which *Saxegothaea* rose, retaining the primitive male gametophyte and something of the cone structure, but becoming specialized in its female gametophyte and its metabolism. If an archegonial complex arose separately as it probably did in the Cupressaceae and the Taxodiaceae, it may well have arisen also in the Podocarpaceae.

In general, therefore, it is suggested that we can visualize the reproductive stages of the primitive podocarp. It would seem to have been characterized by the following main points of structure:—(*a*) winged pollen grains with multiple prothallial cells whose nuclei later became free in the tube; (*b*) a body cell which remained in the grain probably over a long resting period and gave rise to two equal male cells; (*c*) a single gynospore mother cell embedded in a tapetum; (*d*) a very thick two-layered gynospore membrane; (*e*) few—2 to 4—large, long, and narrow pointed archegonia, each with a one-tiered neck suspended across it by a membrane, a ventral canal nucleus, protein vacuoles, and a large egg nucleus awaiting fertilization in its upper part; (*f*) the relations as already described of pollen tube and archegonium; (*g*) two sporophytic divisions at the zone of fertilization followed by further divisions at the base to give a 16-nucleate proembryo before wall formation; (*h*) the organization of the walled proembryo as given in Fig. 1 A; (*i*) the very important binucleate phase of the embryonic cells; (*j*) the development of a single embryo with an early and well-marked

secondary suspensor stage and an apical cell of some duration, and possibly (*k*) the shedding of the seed before the embryo became properly organized.

Several morphologically different lines, probably distinct from very early times, were carved out from the primitive complex, but carried with them most of these features, which however underwent further modifications in some cases.

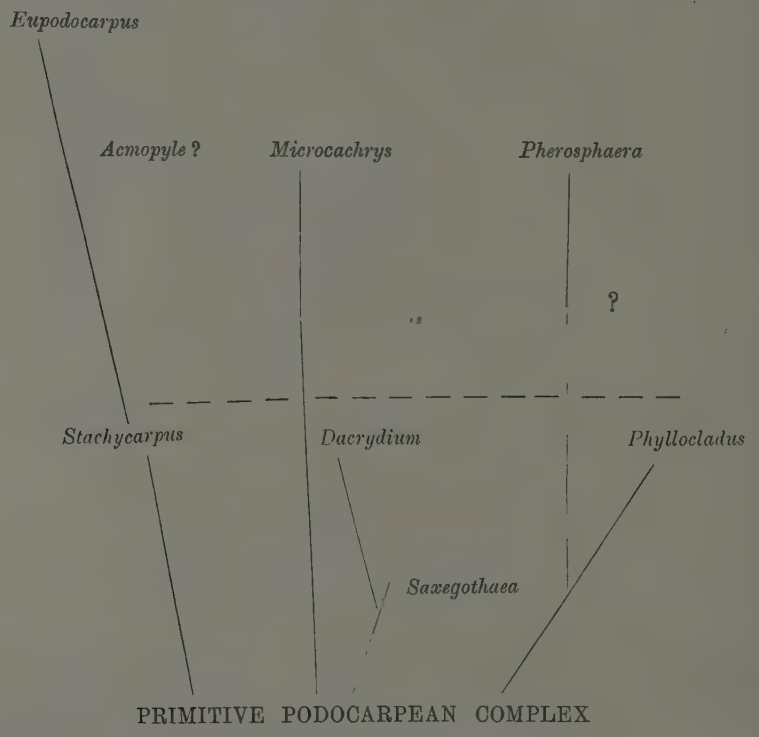

Fig. 4.—Suggested lines of development within the Podocarpaceae.

This is illustrated in the accompanying simple diagram, Fig. 4. In the *Podocarpus* line the simpler *Stachycarpus* section only differs in the possible reduction of one of the male cells, the possible formation of a 32-nucleate stage before wall formation in the proembryo in some species, and the relative importance of the prosuspensors and secondary suspensors; while in the higher members of the line, as in *Eupodocarpus,* both the female gametophyte and the embryo show the modifications to which attention has already been called, including the development of a form of cleavage polyembryony. In the *Phyllocladus* line the features appear almost in full, except possibly for a slight shortening of the archegonia and a reduction in one of the male cells, although both of these points are still a little uncertain. *Pherosphaera* may represent

an extreme development in this line. A third line is shown by *Saxegothaea,* which only differs from the primitive type in a lack of wings on the grain, which is a minor point to be discussed elsewhere, and is related to its special pollination mechanism. Possessing also a distinct cone, this genus must be considered nearest to the basal type, although most probably its general cone morphology exhibits marked reduction from the original strobilus. Very close to this line, and most probably merely an offshoot from it, lies *Dacrydium,* which has also retained, as far as we know, all the features except the development of its peculiar determinate polyembryony, a development which, it has been shown, can arise directly from recognized stages in *Saxegothaea. Microcachrys* is provisionally placed as a special line, arising close to the *Saxegothaea* one and strongly modified, as the higher podocarps and *Pherosphaera* are, but in a manner quite its own.

These conclusions, derived from a comparison of the gametophytes and embryos, agree in general with those of many other writers on the podocarps, particularly perhaps in considering *Saxegothaea* a relatively primitive genus; but in many cases suggestions concerning the relationships of certain genera are coloured by the writer's interpretation of cone morphology. Thus Stiles, influenced by a certain interpretation of cone structure, considers that two main lines arose from the primitive type, one leading to *Saxegothaea* and the other to *Pherosphaera.* The latter line led only to *Phyllocladus.* From the *Saxegothaea* type two branches arose, one leading through *Microcachrys* to *Dacrydium,* and the other to *Podocarpus* through *Stachycarpus.* It is clearly difficult to reconcile the idea of a *Pherosphaera-Phyllocladus* line and a *Saxegothaea-Microcachrys-Dacrydium* line with the developmental history; while the very definite genus *Podocarpus* cannot have arisen from *Saxegothaea,* as the strobili are too diverse, both rather are distinct lines from an earlier type. It is even more difficult to reconcile Sinnott's suggestion that *Eupodocarpus* is the primitive podocarp from whose type the other genera arose. Until some common interpretation of the cone morphology, based on a recognized fossil record, is available it seems safer, in this group at least, to use the known facts of development; and, also, when genera are definitely distinct, to look upon them as examples of different developmental lines rather than to endeavour forcibly to link them up too closely on smaller points of structure of whose importance we are still ignorant, since, in the conifers, we are mainly dealing, in all the Families, with survivors of many different lines of descent, and we are still not in a position to distinguish the important criteria for phylogenetic comparison from those of little value.

Nothing can be gained at present by an attempt to relate the podocarps to the other coniferous Families, as we are not yet in a position to do so. Comparisons between, say, *Pinus, Araucaria, Podocarpus* and *Taxus* are of little value. These and the others are the specialized *living* genera. We could really only compare the primitive members, say the early Mesozoic members, of the various tribes. If we had some knowledge of the nature of the primitive

podocarpean cone from which forms as diverse as *Podocarpus* and *Phyllocladus* were derived, coupled with knowledge such as that outlined in this paper on the probable nature of the primitive gametophytes and embryo, then we could perhaps compare the primitive podocarp with the primitive araucarian, and so on. This cannot yet be done. Similarities can be recognized, but few have any real value. Similarities, for example, between *Saxegothaea* and *Agathis* in ovular structure or in vascular supply to the cone scales need not mean affinity, but merely similar changes from the very early prototypes; and opinions on these matters, without full knowledge of the past, can be little more than speculation. An analysis of this problem may be attempted in the near future, but, for the present it may be said, that, on the basis of their prototypic female gametophyte and embryo, the podocarps seem to show no such really close resemblances to any one of the other Families as to suggest a definite relationship with it rather than with any other.

Summary.

1. In normal embryogeny the 16-cell stage shows a tier of free nuclei (2–5), a tier of prosuspensors (3–5), and a lower group of embryonic cells (6–10) which become binucleate.

2. During prosuspensor elongation the binucleate cells become uninucleate and further divide. A secondary suspensor of long tubular cells is formed, the other cells giving a single embryo, the early stages of whose development usually show an apical cell.

3. Exceptionally, various lobed and double embryonic cell groups occur carried on a common secondary suspensor. This may result, rarely, in an unusual type of polyembryony. The origin of the lobing is indicated, and it is shown that the determinate cleavage of *Dacrydium* may be derived from it.

4. This embryogeny, and also the gametophytes, of *Saxegothaea* are compared with those in other podocarps and discussed.

5. A binucleate phase in the embryonic cells is an important criterion of podocarpean affinity.

6. *Phyllocladus* is definitely a podocarp.

7. The relationships of the genera are considered, and a scheme presented. It is shown that the prototypic gametophyte and embryo have passed from the primitive podocarpean stock into at least three distinct lines, in which subsequent modifications occurred.

8. Simple embryony seems primitive in the podocarps, and from it two distinct types of polyembryony have arisen. Polyembryony is probably derivative in other conifers also.

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No. 12.

"STUDIES IN PEAT."

Part 5.

EXTRACTION OF PEAT WITH AZEOTROPE-LIKE PETROLEUM MIXED SOLVENTS.

By J. REILLY, D. F. KELLY, and J. DUFFY.

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In Part 4 (Sci. Proc. Royal Dublin Soc., **19**, 441, 1930) it was shown that acetone extracted appreciable amounts of a wax-like material from certain types of Irish peat. An investigation has also been made on the yields and properties of the wax-like extraction products obtained from peat by various pure solvents and azeotropic mixtures of solvents (Reilly, Kelly, and Ryan, J. Soc. Chem. Ind., **56**, 231 T, 1937). It has been found that a number of solvents other than petroleum spirits also give equally high or even higher yields of solid wax-like products compared with acetone, e.g., a mixture of benzene and n-propyl alcohol (the azeotrope) gives a yield of the order of 15 per cent. (calculated for the anhydrous peat). In these extractions small amounts of substances of a pitch- and tar-like nature are also extracted, and the crude solid extract leaves a pitch-like residue when treated with white spirit. With petroleum spirits alone the yields on extraction in a soxhlet are generally lower than with acetone, but the extract is completely soluble in white spirit, and the product is a much purer wax. It has been found also that the addition to the petroleum spirits of other organic liquids (i.e., certain alcohols) gave solvent mixtures which also extracted large yields of waxes from peat. In this work the extraction products obtained with petroleum spirits and with mixtures of the spirits with alcohols have been examined. It has been found that for certain proportions the petroleum alcohol mixtures approach the behaviour of azeotropes when used as solvents (cf. Reilly *et al.*, J. Soc. Chem. Ind., **56**, 231 T, 1937). The temperatures of the distillation ranges of the petroleum spirits were found to be lowered. In some cases the mixtures distilled at practically constant temperatures, and in others the temperature ranges were within narrow limits for the greater part of the distillation of the solvent mixtures.

The advantages that azeotropic mixtures (Reilly *et al., loc. cit.*) possess when used as solvents apply to these mixtures of petroleum spirits and alcohols.

Although the petroleum spirits consist of a mixture of substances, yet the mixtures with alcohols behave sufficiently like pure azeotropes of maximum vapour pressure (e.g., approximately constant boiling temperature) to justify further examination from this point of view. Accordingly, not only have the waxes extracted by the petroleum spirit alcohol mixtures been examined, but the behaviour on distillation of the solvent mixtures has also been recorded.

When using solvent mixtures in an extraction plant it is necessary that the composition of the solvent circulating continuously in the different parts (peat container, "wax" still, solvent storage tanks, etc.) of the plant should be approximately constant. Portions of the solvent mixture are being continuously boiled from the still. The composition of solvent in contact with the material being extracted will, therefore, vary from moment to moment if the operation be carried out continuously unless the mixture consists of, or approximates to, an azeotrope. Solvents of an azeotropic nature are desirable in rotary extraction plants which have auxiliary stills in addition to the "wax" still, i.e., the still from which the wax is run off.

In this case also it may be mentioned that a wide latitude, if strict azeotropic conditions be not desired, appears to be allowable in the composition of solvent mixture in contact with the peat. For example, with relatively small amounts of alcohol (e.g., 10 per cent.) present with the petroleum spirits a considerable increase in the yield of wax (over that obtained by pure petroleum liquids themselves) is secured. Further, the lowering of the distillation range of the petroleum spirit also offers advantages.

The petroleum spirits used in the present work consisted of various commercial spirits. They are designated as Y1, Y2, Y3, and Y4, and have the following properties:—

Reference No.	Y 1	Y 2	Y 3	Y 4
Sp. Gr.	0·751	0·728	0·800	0·710
Initial B. Pt.	40°	90°	104°	70° C.
Final B. Pt.	195°	100°	117°	90° C.
Aromatic Content	8 %	10 %	45 %	3 %

An examination of the yields and properties of waxes extracted from peat using mixtures of these petroleum spirits with the alcohols methyl, ethyl, isopropyl, and n-propyl has now been made. For preliminary purposes the peat dried for one hour at 100° C. was extracted in laboratory soxhlet apparatus to exhaustion, the usual time for complete extraction being 12 hours. The yields and properties of the waxes obtained using anhydrous petroleum spirit alcohol mixtures (made up of the different amounts of petroleum spirits Y1, Y2, Y3, and Y4 with the different alcohols in turn) were determined in

each case. In all, 16 different sample mixtures were tested. The properties are summarised in line 1, Table 1. The moisture content of air-dried peat is about 25 per cent., and it, therefore, appeared advisable to examine the effect of water on the extraction of peat with the petroleum alcohol solvents. Line 2 gives the limits of the properties of the waxes obtained using mixtures containing water as a third constituent. In this case the limits given are for

Table I.

	Solvent.	% Yield of extraction dry peat.	Saponification value	Acid value.	Ester value.	Iodine value.	Ether solubility.	White Spirit solubility.	M. Point.
1.	Anhydrous Petroleum-Alcohol Mixtures.	10–14	149–190	50–72	98–133	27–44	36–48	86–100	67–71° C.
2.	Petroleum-Alcohol Mixtures containing water.	11–15	143–180	47–81	85–124	30–35	37–57	80–98	67–71° C.
3.	Pure Petroleum Spirits.	6–8	71–133	35–48	36–82	29–46	36–48	100	65–70° C.

eight samples. For comparative purposes the yields and properties of the waxes obtained using the four petroleum solvents alone were also ascertained, and the range of the yields and properties are given in line 3. The yields and properties (which are not appreciably interfered with by the presence of water in the solvent mixtures) of the petroleum alcohol waxes extracted are not individually recorded. The limits obtained are given in Table 1. The proportions of the spirits Y1 and Y3 with the different alcohols (taken in turn) used in the extraction experiments are recorded later in the paper, the proportions here being the same as those used in the distillation experiments. For the spirits Y2 and Y4 the amounts of these used in the mixtures with the different alcohols (again taken in turn) varied from 40–60 per cent. Experiments carried out, using amounts of alcohol in the mixtures as low as 10 per cent., again gave waxes in yield and properties similar to that of line 1, Table 1.

The constants for the waxes were determined by the usual analytical procedure, the melting point being determined by open capillary tube methods. The methods used for the ether and white spirit solubilities have been described by Reilly *et al.* (*loc. cit.*).

The alcohols, methyl, ethyl, and n-propyl, form constant boiling-point mixtures of minimum boiling point with various hydrocarbons. It is for this reason that petroleum alcohol mixtures show "alcohol flats" in their distillation curves. To find mixtures of the different petroleum spirits and alcohols which gave appreciably flat curves trial distillations were first carried out. For this

purpose the B.S.I. distillation apparatus No. 658–1936 was employed. The mixtures which gave flat or nearly flat curves were then used as solvents in the extraction of waxes from peat. It is of importance to note that three of the petroleum spirits, viz., Y1, Y2, and Y4, with methyl alcohol give two-phase liquid mixtures, i.e., the solvents are partially miscible. Such mixtures may also be used in solvent extraction processes (cf. Reilly *et al., loc. cit.*).

The existence of completely flat distillation curves in the case of petroleum spirits Y1, Y2, and Y3 could not be found for any proportion of the different alcohols, but the curves exhibited definite lowering of boiling range and had partial "flats." This is well illustrated by the effect of the addition of the alcohols to petroleum spirit Y1, which itself possesses a relatively wide distillation temperature range. The "flats" observed follow the well-known "flats" for petrol ethyl alcohol mixtures, as used for internal combustion engines. Curve A in Fig. 1 is that of Y1 spirit alone. Curve B is that of the mixture of Y1 spirit and 50 per cent. by volume of ethyl alcohol. It will be noticed that addition of this amount of the alcohol caused the amount of material distilling below 80° C. to increase from 18 per cent. to 80 per cent. approximately. Curve C is that of a 50 : 50 mixture of Y1 spirit and isopropyl alcohol. The curve exhibits a long "alcohol flat" in this case also. Curve D is a similar one for this petrol and n-propyl alcohol. The amount of material distilling below 95° C. increases from 28 per cent. to 80 per cent. on addition of this amount of the n-propyl alcohol. Similar effects were found for the other spirits employed, but the effect is most striking for the Y1 spirit. For

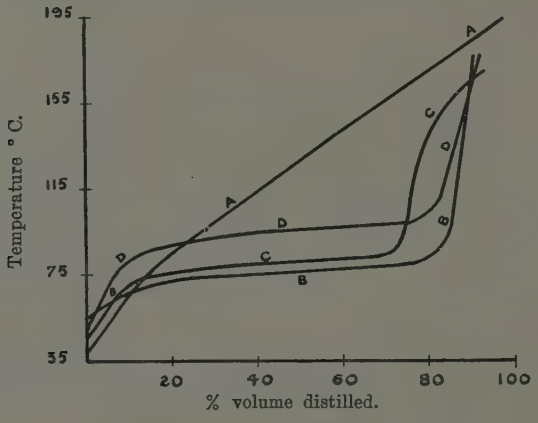

Fig. 1.

example, on distilling Y3 spirit (distillation range 104–117° C.) with 40 per cent. of ethyl alcohol, it was found that 65 per cent. of the mixture distilled over between the temperatures 72° C. and 75° C. Increasing the percentage of alcohol increased the length of the "alcohol flat." Similar results were

obtained with the alcohols methyl, isopropyl, and n-propyl. It was found that using in each case the percentage (by volume) of alcohol specified below with Y3 spirit a practically horizontal distillation curve resulted, with appreciable decrease in the boiling temperatures. The results are shown graphically in Fig. 2. It should be noted that in this work "percentage volume distilled" relates the volume distilled to the volume originally taken, and not to the total distillate obtained as in routine petroleum-distillation tests. Figures on the latter basis would give enhanced "flats," but these would have little significance here. Again referring to Fig. 2, Curve I is that of the petroleum spirit alone. Curve III is that of the mixture of the petroleum spirit plus 70 per cent. of isopropyl alcohol. Curves IV and V show the effect of the addition of 60 per cent. of the alcohols ethyl and methyl, respectively. It will be observed that these mixtures approximate closely to a mixture of two pure liquids which form a constant boiling mixture of minimum boiling point as is also the case with benzene n-propyl alcohol (cf. Curve II).

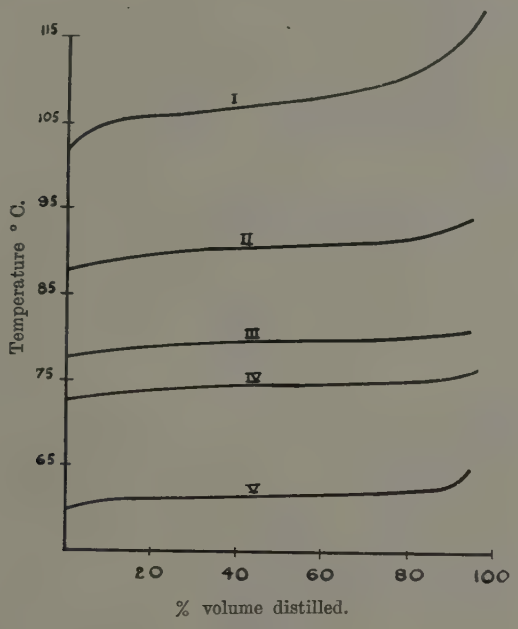

Fig. 2.

The behaviour of these mixtures has also been examined on distillation in the presence of water and of wax (previously extracted from peat by the particular solvents in question). This is of importance, as in technical scale plant wax and water are present in the still from which solvents are being

distilled. The results in the test distillations have been recorded graphically. In Fig. 3, Curve A represents the distillation of a mixture containing 60 per cent. methyl alcohol and 40 per cent. Y3 spirit to which was added (before the distillation) 2·5 per cent. of "Y3 spirit methyl alcohol" wax. It will be seen that the presence of the wax has no appreciable effect on the distillation temperature range of the pure solvent mixture shown by dotted Curve A_1. Curve B represents the addition of 2 per cent. water to the above mixture of solvent and wax. The initial and final boiling points are slightly raised by the addition of this amount of water. Curve C is that for a mixture of Y3 spirit and ethyl alcohol (40 : 60—by volume) containing at the beginning of the distillation 2·5 per cent. (by weight) of "Y3 spirit ethyl alcohol" wax. Here again the presence of the wax has little effect on the distillation range of the original solvent mixture (cf. dotted Curve C_1). Curve D represents the distillation of the above mixture with, in addition, 5 per cent. of water. Here the initial boiling point is slightly lower than that of the preceding mixture.

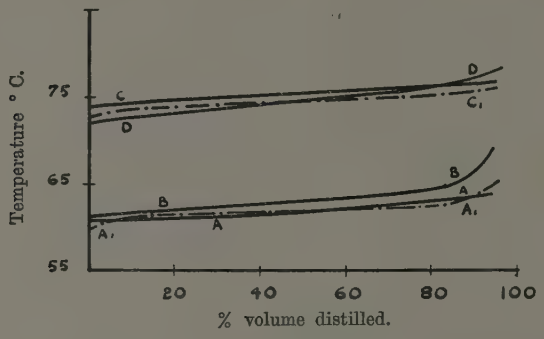

FIG. 3.

Curve E, Fig. 4, shows the behaviour of a mixture of 30 per cent. of Y3 spirit and 70 per cent. isopropyl alcohol to which was added 2·5 per cent. of "Y3 spirit isopropyl alcohol" wax. Curve F is for the above mixture plus 5 per cent. of water. The boiling range of 95 per cent. of this mixture is below that of the preceding mixture, while the final boiling point is slightly higher. Curve E_1 is that for the solvent mixture alone. Curve G is that for a mixture of 50 per cent. of Y3 spirit with 50 per cent. of n-propyl alcohol containing at the beginning 2·5 per cent. of "Y3 spirit n-propyl alcohol" wax. Curve H shows the effect of the addition of 2 per cent. of water to the above mixture. The initial boiling point is lowered 9° C. by the presence of this amount of water, while the final boiling point is slightly higher. Curve G_1 is that of the solvent mixture alone. It will be noted that the presence of (*a*) wax or (*b*) wax and water in the solvent mixtures does not inordinately interfere with the constancy of the distillation range of the mixtures.

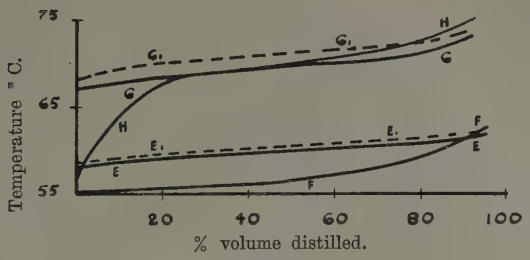

Fig. 4.

The peat used in these experiments consisted of a well-sampled specimen secured from a consignment of air-dried peat from the Coraffrin bog (Ordnance Survey Sheet No. 87), Co. Donegal.

Summary.

The solvent extraction of wax-like products from Irish peat using petroleum spirits alone and mixtures of these spirits with alcohols has been studied. Petroleum spirits used alone give low yields in comparison with other solvents. It has been found, however, that the addition of alcohols to the petroleum spirits gives much increased yields of the extraction products. The yields and properties of the waxes are recorded. The behaviour of these petroleum alcohol mixtures on distillation has also been examined. It has been found that the addition of alcohols to the petroleum spirits has the additional advantage of lowering the distillation temperatures of the mixtures, while in some cases mixtures have been found which approximate on distillation to an azeotropic mixture of two pure solvents (of maximum vapour pressure). The presence of wax and/or water in the solvent mixtures does not seriously interfere with the azeotrope-like property of the mixtures.

The authors wish to express their thanks to the Industrial Research Council for facilities to conduct this research and for permission to publish.

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No. 13.

STUDIES ON THE ULTIMATE COMPOSITION OF BIOLOGICAL MATERIAL.

Part III.—Glandular Tissues.

By ROBERT PRESS and WILLIAM ROBERT FEARON.

[Read March 21. Published separately May 25, 1939.]

In extending the applications of spectrographic analysis to the study of the composition of the higher animals, it is desirable to restrict the field to particular types of tissue in order to see if there has been any accumulation of biological elements or characteristic alteration in the distribution of the elements that are represented in the blood and other circulating fluids. Because of their physiological importance and uniformity of function, the ductless glands were selected for the present investigation, and analyses have been made of pituitary, pineal, adrenal, and testicular tissue of the horse, cow, sheep, and pig, together with an analysis of liver tissue for the purpose of comparison.

The procedure adopted was similar to that previously described (Webb and Fearon, 1937; Webb, 1937). The dried and fat-extracted material was ashed in a fused quartz crucible enclosed in a similar crucible, and placed in an electric furnace, the heat dissipation of which could be "stepped" as required. The ash was then thoroughly triturated with an equal weight of spectroscopically pure ammonium sulphate, and samples of approximately 3 mg. were transferred to the lower electrode of the carbon or silver arc, which was connected with the positive lead. The voltage supply was 100, which fell on striking to 35 volts, the current being 4 amps.

The precautions taken against experimental contamination have been previously described, an additional safeguard adopted being the use of a chimney over the arc housing to remove all vapours from the room in which the experiments were carried out, and thus avoid atmospheric contamination. As in the previous work, a Hilger medium quartz spectrograph (E 488) was employed.

Results.

The following elements were detected in all specimens examined:—sodium, potassium, calcium, magnesium, iron, copper, manganese. Lead, aluminium, and rubidium occurred sporadically; zinc was detected only in one tissue. No indication was obtained of the presence of boron, barium, cadmium, cobalt, chromium, lithium, molybdenum, nickel, or antimony. Strontium occurred in traces in five of the specimens.

TABLE 1.

Elementary Composition of Glandular Tissue.

Element.	Al	Cu	K	Na	Mn	Sn	Zn	Sr	Ca	Mg	Fe	Pb	Si	Rb
Pituitary,														
Sheep	(+)	+	++	+++	+	–	+	(+)	+++	+++	+++	+	+	+
Horse	(+)	+	++	+++	+	–	–	(+)	+++	+++	+++	(+)	+	–
Pig	–	+	++	+++	+	(+)	–	–	+++	+++	+++	(+)	(+)	–
Testis,														
Pig	(+)	+	++	+++	(+)	–	–	–	+++	+++	+++	(+)	+	–
Adrenal,														
Sheep	(+)	+	+++	+++	(+)	–	–	–	+++	+++	+++	+	+	–
Horse	–	+	+++	+++	+	–	–	(+)	+++	+++	+++	(+)	+	–
Ox	(+)	+	+	+++	+	(+)	–	(+)	+++	+++	+++	+	(+)	–
Pineal,														
Sheep	(+)	+	++	+++	(+)	(+)	–	(+)	+++	+++	+++	(+)	+	(+)
Ox	+	+	+	+++	+	(+)	–	–	+++	+++	+++	+	+	–
Liver,														
Horse	+	+	+++	+++	+	–	–	–	+++	+++	+++	(+)	(+)	(+)

No indication could be obtained of the presence of the following elements in any of the preparations:—B, Ba, Cd, Co, Cr, Li, Mo, Ni, Sb.

In the accompanying table, the entry (+) denotes that the particular element is just detectable in the spectrogram. As the limits of detectability differ for different elements, the significance of (+) was ascertained by the use of standard powders containing known concentrations of the different elements. Three-milligram samples of each powder were placed in the arc, and the spectrogram compared with that yielded by the same weight of the mixture of tissue ash and ammonium sulphate.

In the standard powders, the concentrations of Al, Ba, B, Co, Cr, Na, and Sn was such that (+) represented an amount of each element that was less than $0{\cdot}27\gamma$, while + may be taken to represent an intensity at least one order higher, or about $2{\cdot}7\gamma$, in the 3 mgm. sample.

For Cd, Cu, Fe, Mn, Sr, P, and Pb, in the standard powders, (+) represents less than $0{\cdot}027\gamma$; while for Ni, Sb, and Zn, (+) represents less than $2{\cdot}7\gamma$.

Expressing these results in terms of the tissue ash: for Al and Sn, (+) represents less than 0·018 per cent., and + represents at least 0·18 per cent. For Mn, Sr, and Pb, (+) represents less than 0·002 per cent., and for Zn, (+) represents less than 0·18 per cent.

It is interesting to compare these results with the previously reported analyses of glandular tissue carried out by Sheldon and Ramage (1931), using a flame spectrum technique. During their survey of human tissue, they report the constant presence of Na, K, Ca, Mg and Fe, and the micro-constituents, Cu, Mn, Rb, and Ag.

TABLE 2.

(Compiled from data of Sheldon and Ramage, 1931.)

Percentage Composition of Human Glandular Tissues.

Element.	Cu	Mn	Rb	Ag
Pituitary	0·002	0·0008	(+)	
Testis	(+)	(+)	0·0016	(+)
Adrenal	+	(+) to 0·002	+	(+)
Liver	(+) to 0·03	(+) to 0·002	(+) to 0·004	(+)

As regards copper and manganese, these findings are in accordance with our observations. Our frequent failure to detect rubidium, which Sheldon and Ramage regard as "almost invariably present in tissues," is probably due to the greater sensitivity of the flame technique with regard to this particular metal. Zbinden (1930), using the arc spectra, has also failed to detect rubidium in a variety of biological material examined. On the other hand, the arc technique has enabled us to detect aluminium, tin, zinc, strontium, and lead in many of the specimens, although we have no reason to regard their occurrence as being biologically significant.

We acknowledge with gratitude the kindness of Dr. A. S. Parkes, F.R.S., who has provided us with a supply of acetone-extracted tissues for examination, and the advice and assistance of Professor R. W. Ditchburn throughout the work. The spectrographic preparations and analyses have been entirely carried out by one of us (R.P.) who is in receipt of a research grant from the Irish Hospital Trust.

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No. 14.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1938.

[Read APRIL 18. Published separately MAY 26, 1939.]

THE issue of radon tubes for therapeutic purposes has slightly exceeded that for the previous year, 1,582 tubes containing 11,202 millicuries having been sent out in 1938, as compared with 1,526 tubes containing 10,110 millicuries in 1937.

Mr. John Nash was awarded the Radium Exhibition falling vacant during the year.

Returns received from the chief users record the treatment of the following cases with radon or radium element during the year:—

Malignant Cases.

Dr. Oswald Murphy, St. Vincent's Hospital	113
Dr. Oliver Chance	51
St. Anne's Hospital, Dublin	292
City of Dublin Skin and Cancer Hospital	34
County and City Infirmary, Waterford	18
National Maternity Hospital, Dublin	6
Dr. Steevens' Hospital, Dublin	2
Adelaide Hospital, Dublin	1
Coombe Hospital, Dublin	1
Total, ...	518

Non-malignant Cases.

Dr. Oswald Murphy	85
Dr. Oliver Chance	35
St. Anne's Hospital, Dublin	12
Total, ...	132

The returns show that for the cases of malignant disease first seen during 1938 the numbers treated by the different methods were as follows:—

Number of cases seen	1,202
„ „ treated	987
„ „ „ by Surgery alone	76
„ „ „ „ Radium alone	323
„ „ „ „ X-rays alone	442
„ „ „ „ Surgery and Radium ...	25
„ „ „ „ Surgery and X-rays ...	51
„ „ „ „ Radium and X-rays ...	67
„ „ „ „ Surgery, Radium, and X-rays	3

The following reports have been received summarising the results of treatments carried out with radium or radon (or in some cases X-rays) during the year, and the later results of treatments carried out in former years.

Report by Oswald J. Murphy, M.B., B.Ch.,

St. Vincent's Hospital, Dublin.

During the year under review I am glad to state that on an average the cases treated arrived in earlier stages of malignancy than in the previous year; however, there is still great room for improvement.

One unfortunate patient had been treated for nearly six months by a peroxide mouthwash for a large malignant ulcer involving middle and posterior thirds of the tongue!

During the year one case of cancer of the tongue treated in 1931 and listed as having died in 1935 (an untraced case) came and reported fit and well—there is no evidence of any trouble.

MALIGNANT CASES TREATED DURING 1938.

Number treated by Surgery and Radium	4
Number treated by Radium only	109
	113

	Seen.	Treated.
Carcinoma of the Ear	3	3
„ „ Lip	6	6
„ „ Cheek	2	2
„ „ Parotid	2	2
„ „ Tongue	9	9
„ „ Floor of Mouth	4	4
„ „ Antrum	1	1
„ „ Tonsil	1	1
„ „ Breast	1	1
„ „ Breast (Secondaries)	6	6
„ „ Penis	1	1
„ „ Bladder	1	1
„ „ Uterine Cervix	7	7
„ „ Uterine Body	3	3
„ „ Skin Surfaces	36	35
Rodent Ulcers	29	29
Sarcoma of Cheek	1	1
Sarcoma of Arm	1	1
Total, ...	114	113

Results of Treatment. Malignant Cases.

Carcinoma of the Ear, 3.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	3	2	1	–	–	–
Glands ...	0	–	–	–	–	–

Carcinoma of the Lip, 6.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	6	6	–	–	–	–
Glands ...	0	–	–	–	–	–

Carcinoma of the Cheek, 2.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	2	2	–	–	–	–
Glands ...	0	–	–	–	–	–

Carcinoma of the Tongue, 9.

Anterior Third, 2.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	1	–	1	–	–	–
Glands ...	1	–	1	–	–	–

Middle Third, 6.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	2	2	–	–	–	–
Glands ...	4	–	3	–	1	–

Posterior Third, 1.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	–	–	–	–	–	–
Glands ...	1	–	1	–	–	–

Carcinoma of the Floor of the Mouth, 4.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	2	1	1	–	–	–
Glands ...	2	–	1	–	1	–

Carcinoma of the Uterine Cervix, 7.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
Group I ...	3	2	1	–	–	–
Group II ...	3	2	1	–	–	–
Group III ...	1	–	1	–	–	–

Carcinoma of the Skin Surfaces, 35.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	32	14	13	1	3	1
Glands ...	3	1	2	–	–	–

Rodent Ulcers, 30.

No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
30	25	2	–	3	–

Sarcoma of Cheek, 1.

This case died after 6 months.

Sarcoma of the Arm.

Has only just finished his treatment.

NON-MALIGNANT CASES.

	Seen.	Treated.
Exophthalmic Goitre	2	2
Hodgkin's Disease	5	5
T.B. Cervical Glands	8	8
T.B. Peritonitis	7	7
Uterine Hæmorrhages	1	1
Angiomata	14	14
Lupus Vulgaris	4	4
Lupus Erythematosus	5	5
Keloids	4	4
Dupytron Contraction	1	1
Ludwig's Angina	1	1
Pruritis Vulvae	1	1
Warts	32	32
	85	85

SUMMARY OF SOME OF THE MORE IMPORTANT GROUPS OF MALIGNANT CASES TREATED DURING THE SEVEN-YEAR PERIOD, 1931–1937.

Carcinoma of the Ear.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	0 ...	0	0	0	0	0	0	0
1932	0 ...	0	0	0	0	0	0	·
1933	2 ...	2	1	1	1	1	·	·
1934	4 ...	3	3	3	3	·	·	
1935	4 ...	3	2	2				
1936	5 ...	5	4					
1937	3 ...	3						

Carcinoma of the Cheek.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1933	4 ...	4	3	3	2	2	.	.
1934	6 ...	5	5	4	4			
1935	4 ...	3	3	3				
1936	3 ...	3	3					
1937	4 ...	4						

Carcinoma of the Tongue.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	4 ...	2	1	1	1	1	0	0
1932	8 ...	4	3	3	2	1	0	
1933	3 ...	2	1	1	0	0		
1934	5 ...	2	2	1	1			
1935	9 ...	5	2	2				
1936	10 ...	6	4					
1937	7 ...	6						

Carcinoma of the Lip.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	3 ...	2	2	1	1	1	0	0
1932	7 ...	5	4	4	3	2	2	
1933	6 ...	5	4	4	2	2		
1934	7 ...	5	5	3	3			
1935	8 ...	6	2	2				
1936	8 ...	7	6					
1937	6 ...	6						

Carcinoma of the Palate.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	2 ...	1	1	1	1	0	0	0
1932	4 ...	3	2	0	0	0	0	
1933	2 ...	2	2	2	2	1		
1934	1 ...	1	1	0	0			
1935	2 ...	1	1	1				
1936	1 ...	1	1					
1937	1 ...	1						

Carcinoma of the Tonsil.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	2 ...	1	1	0	0	0	0	0
1932	3 ...	2	2	1	0	0	0	
1933	2 ...	1	1	1	1	1		
1934	5 ...	3	2	1	0			
1935	3 ...	2	1	1				
1936	1 ...	1	1					
1937	1 ...	1						

Carcinoma of the Larynx and Pharynx.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	4 ...	1	0	0	0	0	0	0
1932	5 ...	1	1	1	0	0	0	
1933	1 ...	1	0	0	0	0		
1934	2 ...	2	0	0	0			
1935	3 ...	2	1	1				
1936	1 ...	0	0					
1937	0 ...	0						

Carcinoma of the Breast (Primary).

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	4 ...	2	1	1	1	0	0	0
1932	0 ...	–	–	–	–	–	–	
1933	2 ...	1	1	0	0	0		
1934	2 ...	1	1	1	1			
1935	5 ...	3	1	1				
1936	4 ...	3	2					
1937	1 ...	1						

Carcinoma of the Breast (Secondaries).

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	6 ...	2	1	0	0	0	0	0
1932	0 ...	–	–	–	–	–	–	
1933	11 ...	2	1	1	1	0		
1934	14 ...	4	1	0	0			
1935	7 ...	3	1	0				
1936	8 ...	5	2					
1937	6 ...	3						

Carcinoma of the Cervix Uteri.

Year of Treatment.	No. Treated.	Alive after 1	2	3	4	5	6	7 years.
1931	3 ...	1	1	1	1	1	0	0
1932	8 ...	5	4	4	3	1	0	
1933	3 ...	2	2	1	1	1		
1934	3 ...	3	2	2	2			
1935	5 ...	4	2	2				
1936	7 ...	5	5					
1937	7 ...	5						

Report by Oliver Chance, M.B., B.Ch., D.M.R.E.,

Richmond, Sir P. Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals.

During 1938 most of the cases requiring radiation seen in the above hospitals were referred to St. Anne's Hospital for treatment. The bulk of the cases included below were either private patients or else suffering from conditions for which they could be treated as out-patients.

Malignant Cases (94).

Number of cases of all types of malignant disease first seen during 1938	113
Number of cases of all types of malignant disease treated during 1938	99
Number of cases of all types of malignant disease treated by Surgery alone in 1938 ...	5
Number of cases of all types of malignant disease treated by Radium alone in 1938 ...	41
Number of cases of all types of malignant disease treated by X-rays alone in 1938 ...	40
Number of cases of all types of malignant disease treated by Surgery and Radium ...	3
Number of cases of all types of malignant disease treated by Surgery and X-rays ...	3
Number of cases of all types of malignant disease treated by Radium and X-rays ...	7
Number of cases of all types of malignant disease treated by Surgery, Radium, and X-rays	–

Total Malignant Cases treated by Radiation.

Rodent Ulcers. Seen 26. Treated, 25. Unsuitable, 1.
Well, 19. Still under treatment, 3. Untraced, 2. Unimproved, 1.

Carcinoma of Skin. Seen 14. Treated, 13. Unsuitable, 1.
Well, 9. Still under treatment, 1. Untraced, 3.

Carcinoma of Lip. Seen 11. Treated, 8. Treated by Surgery, 1. Unsuitable, 2.
Well, 5. Still under treatment, 1. Untraced, 2.

Carcinoma of Tongue. Seen 8. Treated, 7. Unsuitable, 1.
Well, 5. Still under treatment, 1. Unimproved, 1.

Carcinoma of Floor of Mouth. Seen 3. Treated, 2. Unsuitable, 1.
Dead, 1. Untraced, 1.

Carcinoma of Tonsil. Seen 3. Treated, 2. Unsuitable, 1.
Unimproved, 1. Untraced, 1.

Carcinoma Larynx (*Intrinsic*). Seen 1. Treated, 1.
Well, 1.

Carcinoma Larynx (*Extrinsic*). Seen 9. Treated, 8. Unsuitable, 1.
Well, 1. Improved, 2. Unimproved, 3. Dead, 1. Untraced, 1.

Post-Cricoid Carcinoma. Seen 2. Treated, 2.
Dead, 2.

Oesophagus. Seen 2. Treated, 0. Unsuitable, 2.

Carcinoma Breast. Seen 12. Treated, 8. Treated by Surgery, 2. Unsuitable, 2.
(a) *Operable,* 1. Alive, 1.
(b) *Inoperable,* 7. Well, 1. Improved, 2. Unimproved, 1. Untraced, 1.
(c) *Post-Operative recurrence,* 2. Well, 1. Improved, 1.

Carcinoma Cervix. Seen 1. Treated, 1.
Grade III. Well, 1.

Carcinoma Body of Uterus. Seen 5. Treated, 3. Treated by Surgery, 2.
Grade III. Improved, 1. Unimproved, 1. Dead, 1.

Carcinoma Thyroid. Seen 2. Treated, 2.
Improved, 1. Unimproved, 1.

Carcinoma of Middle Ear. Seen 1. Treated, 1.
Untraced, 1.

Carcinoma Bladder. Seen 1. Treated, 1.
Dead, 1.

Carcinoma Penis. Seen 1. Treated, 1.
Well, 1.

Carcinoma Testis. Seen 1. Treated, 1.
Well, 1.

Carcinoma Lung. Seen 2. Treated, 2.
Dead, 2.

Carcinoma Rectum. Seen 5. Treated, 0. Unsuitable, 5.

Sarcoma (Various). Seen 6. Treated, 6.
Well, 1. Improved, 2. Unimproved, 1. Dead, 2.

NON-MALIGNANT CASES (treated with Radium or Radon) (35).

Hodgkin's Disease. 3.
Improved, 2. Dead, 1.

Toxic Goitre. 10.
Well, 3. Improved, 2. Unimproved, 2. Still under treatment, 1. Untraced, 2.

Naevi. 6.
Well, 3. Improved, 2. Still under treatment, 1.

Metropathia Hæmorrhagica. 6.
Well, 4. Unimproved, 1. Untraced, 1.

Adenoma Prostate. 1.
Well, 1.

Tubercular Peritonitis. 6.
Well, 3. Improved, 1. Dead, 2.

Keloids. 3.
Well, 2. Improved, 1.

Report from St. Anne's Hospital, Dublin.

Radiologist: Dr. Oliver Chance, M.B., D.M.R.E.

Number of cases of all types of malignant disease seen during 1938	675
Number of cases of all types of malignant disease treated during 1938	519
Number of cases of all types of malignant disease treated by Surgery alone in 1938 ...	54
Number of cases of all types of malignant disease treated by Radium alone in 1938 ...	131
Number of cases of all types of malignant disease treated by X-rays alone in 1938 ...	253
Number of cases of all types of malignant disease treated by Surgery and Radium ...	10
Number of cases of all types of malignant disease treated by Surgery and X-rays ...	20
Number of cases of all types of malignant disease treated by Radium and X-rays ...	48
Number of cases of all types of malignant disease treated by Surgery, Radium, and X-rays	3

Record of Cases treated in past Five Years.

In the following tables any patient who could not be traced in March, 1939, is regarded as dead. The figures, therefore, show the crude survival rate. It must be realised that this has little bearing on the percentage of cases who were cured. More than 1 in 6 of the cases are untraced. There is little doubt that many of these are still alive and free from disease. In several groups the number of patients alive in 1939 is actually larger than in the previous year. This is accounted for by the fact that several patients untraced in 1938 have been followed up successfully this year.

It is to be remembered that one in seven of our patients are over 70 years old—two patients treated recently were 91 and 92 respectively—the expectation of life in many cases is, therefore, only a few years.

When the above facts are realised, it will be obvious that the cold figures which follow can give little idea of the true value of radiation, not only in curing disease, but also in relieving symptoms and prolonging life.

Cancer of Lip.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
9	8	7	6	3	3
	12	11	10	6	7
		35	24	22	25
			39	37	32
				58	49

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	1	1	1	1	0
	5	3	3	1	2
		8	7	6	8
			23	20	17
				15	13

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	1	1	1	0	1
	16	9	8	3	1
		16	13	5	5
			7	3	2
				11	7

Cancer of Tongue.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
10	7	5	3	2	2
	20	10	10	4	3
		20	14	10	13
			15	11	7
				16	12

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	2	2	1	0	0
	8	2	0	0	1
		9	8	6	2
			10	10	5
				10	5

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	0	0	0	0	0
	1	0	0	0	0
		0	–	–	–
			2	0	1
				1	1

Cancer of Cheek.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	1	1	1	1	1
	4	4	3	2	1
		3	2	2	2
			2	2	2
				0	–

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	0	0	0	0	0
	2	0	0	0	1
		2	2	2	2
			1	0	1
				0	–

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	0	0	0	0	0
	1	0	0	0	0
		0	–	–	–
			2	0	1
				1	1

Cancer of Alveolus.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	3	1	0	0	1
		2	0	0	0
			2	2	0
				2	2

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	0	–	–	–	–
		1	1	0	0
			4	0	1
				2	1

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	0	0	0	0	0
	4	0	0	0	0
		3	3	0	0
			2	1	0
				1	–

Cancer of Palate.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	1	1	1	1	0
	3	1	0	0	1
		3	2	0	1
			5	4	2
				1	1

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	1	1	1
		4	3	0	0
			1	1	1
				0	–

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	0	0	0	0	0
	2	0	0	0	0
		0	–	–	–
			1	0	0
				2	0

Cancer of Floor of Mouth.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
3	2	2	2	2	1
	2	2	2	2	2
		7	7	5	4
			11	8	6
				7	6

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	0	0	0
		0	–	–	–
			3	2	1
				1	1

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	5	1	0	0	0
		3	3	1	0
			6	1	1
				2	1

Cancer of Fauces.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	2	0	0	0	0
		1	1	1	1
			3	3	3
				6	5

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	0	0	0
		1	0	0	1
			2	2	0
				1	0

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	5	1	0	0	0
		3	3	0	0
			1	0	0
				5	3

Cancer of Tonsil.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	2	0	0	0	0
	9	3	2	0	0
		8	3	3	1
			11	4	0
				3	2

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	1	1	1
		2	2	0	0
			1	1	0
				1	0

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	2	0	0	0	0
	9	3	2	0	0
		8	3	3	1
			11	4	0
				3	2

Cancer of Larynx.

Extrinsic.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	0	0	0	0	0
	10	0	0	0	0
		12	1	1	2
			13	5	1
				12	8

Intrinsic.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	0	–	–	–	–
		1	1	0	0
			1	1	1
				3	2

Post-cricoid.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	0	0	0	0	0
	4	0	0	0	0
		3	0	0	0
			8	0	0
				5	1

Cancer of Breast.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	0	0	0	0	0
	2	2	2	1	1
		2	2	2	2
			5	5	4
				1	1

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	0	0	0	0	0
	2	2	2	1	1
		8	4	4	2
			8	7	2
				7	5

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
3	0	0	0	0	0
	11	7	4	4	4
		19	10	3	1
			13	10	4
				21	12

Post-operative recurrence—Palliative treatment.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	5	4	2	1	1
		12	6	3	3
			9	7	2
				16	11

Post-operative prophylactic treatment.

Alive in:—					March
1934	1935	1936	1937	1938	1939
2	0	0	0	0	0
	6	4	1	1	1
		1	1	1	1
			9	5	5
				8	6

Cancer of Cervix Uteri.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	0	–	–	–	–
		2	2	2	2
			11	10	5
				4	2

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
1	1	1	1	0	0
	5	3	2	1	1
		4	3	2	2
			4	4	4
				9	5

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	5	0	0	0	0
		16	13	3	0
			13	6	5
				23	12

Cancer of Body of Uterus.

Alive in:—					March
1934	1935	1936	1937	1938	1939
4	4	4	4	2	1
	8	3	3	3	4
		7	4	4	5
			2	0	0
				0	3

Cancer of Skin.

Stage 1.

Alive in:—					March
1934	1935	1936	1937	1938	1939
15	13	13	13	8	5
	38	32	31	7	4
		42	39	29	26
			41	34	29
				52	28

Stage 2.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	1	1	0
		2	2	2	2
			4	4	4
				6	2

Stage 3.

Alive in:—					March
1934	1935	1936	1937	1938	1939
0	–	–	–	–	–
	1	1	1	1	0
		2	2	1	1
			2	2	2
				2	2

Rodent Ulcers.

Alive in:—					March
1934	1935	1936	1937	1938	1939
49	31	23	17	13	8
	38	32	31	20	16
		57	48	39	35
			74	56	57
				82	76

NON-MALIGNANT CASES (treated with Radium or Radon).

Naevi, 5; Angiomata, 1; Benign tumour of parotid, 1; Menorrhagia, 4; Uterine fibroids, 1; Total, 12.

TOTAL CASES TREATED WITH RADIUM OR RADON DURING 1938.

Number of new cases treated with Radium:—

Malignant cases	192
Non-malignant cases	6
Skin cases	6
Number of return cases treated	100
Total, ...	304

Radium, 255. Radon, 42. Seeds, 7.

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